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Goeddel et al.

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(54) **MICROBIAL PRODUCTION OF MATURE HUMAN LEUKOCYTE INTERFERONS**

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Related U.S. Application Data

(63) Continuation of application No. 06/703,148, filed on Feb. 19, 1985, now abandoned, which is a division of application No. 06/256,204, filed on Apr. 21, 1981, which is a continuation-in-part of application No. 06/205,578, filed on Nov. 10, 1980, now abandoned, which is a continuation-in-part of application No. 06/184,909, filed on Sep. 8, 1980, now abandoned, which is a continuation-in-part of application No. 06/164,986, filed on Jul. 1, 1980, now abandoned.

(51) **Int. Cl.**[7] **C12P 21/02**

(52) **U.S. Cl.** **435/69.51**; 435/69.1; 435/252.3; 435/252.33; 536/23.52

(58) **Field of Search** 435/69.51, 172.3, 435/243, 252.3, 252.33, 320.1; 536/27, 23.52; 424/85.7

(56) **References Cited**

U.S. PATENT DOCUMENTS

4,289,690	A	9/1981	Pestka	
4,332,892	A	6/1982	Ptashne et al.	
4,342,832	A	8/1982	Goeddel et al.	
4,418,149	A	11/1983	Ptashne et al.	
4,503,035	A	3/1985	Pestka et al.	424/85.7
4,530,901	A *	7/1985	Weissmann	435/69.51
4,801,685	A	1/1989	Goeddel et al.	435/69.51
4,810,645	A	3/1989	Goeddel et al.	435/69.51

FOREIGN PATENT DOCUMENTS

EP	0 001 929	6/1979
EP	6694	1/1980
EP	8030110	4/1980
GB	2007676 A	5/1979
GB	2055382	3/1981

OTHER PUBLICATIONS

Allen, 1982, "Structure and properties of human interferon–αfrom Namalwa lymphoblastoid cells," *Biochem. J. 207*:397–408.

Allen and Fantes, 1980, "A family of structural genes for human lymphoblastoid (leukocyte–type) interferon," *Nature 287*:408–411.

Anfinsen, 1981, "Human interferon," *Interdisciplinary Science Reviews 6*(2):110–118.

Bolivar et al., 1977, "Construction and characterization of new cloning vehicles. II. A multipurpose cloning system," *Gene 2*(2):95–113.

Cavalieri et al., 1977, "Synthesis of human interferon by *Xenopus laevis* oocytes: two structural genes for interferons in human cells," *Proc. Natl. Acad. Sci. U.S.A. 74*(8):3287–3291.

Crea et al., 1978, "Chemical synthesis of genes for human insulin," *Proc. Natl. Acad. Sci. U.S.A. 75*(12):5765–5769.

Goeddel et al., 1981, "The structure of eight distinct cloned human leukocyte interferon cDNAs," *Nature 290*(5801):20–26.

Goeddel et al., 1980, "Synthesis of Human Fibroblast Interferon by E. Coli," *Nucleic Acid Research 8*:4057–74.

Goeddel et al., 1979, "Expression in *Escherichia coli* of chemically synthesized genes for human insulin," *Proc. Natl. Acad. Sci. U.S.A. 76*(1):106–110.

Green et al., 1976, "Isolation and cell–free translation of immunoglobulin messenger RNA," *Arch. Biochem. Biophys. 172*(1):74–89.

Attached are pp. 135–137 of the Main Brief at Final Hearing for Goeddel et al. (including the pages of the record and exhibits cited therein) in Interference No. 101,601, *Goeddel et al. v. Weissmann*, re: Heynecker, Speech at NIH Interferon Workshop, Sep. 8–9, 1980.

Itakura et al., 1977, "Expression in *Escherichia coli* of a chemically synthesized gene for the hormone somatostatin," *Science 198*(4321):1056–1063.

Levy et al., 1981, "Amino acid sequence of a human leukocyte interferon," *Proc. Natl. Acad. Sci. U.S.A. 78*(10):6186–6190.

Mantei et al., *Chemical Abstracts*, 1980, vol. 93, p. 489, Abstract No. 130319s.

Miller, 1979, "Use of recombinant DNA technology for the production of polypeptides," *Adv. Exp. Med. Biol. 118*:153–174.

Nagata et al., *Chemical Abstracts*, 1980, vol. 93, p. 479, Abstract No. 41286m.

Pestka et al., 1987, "Interferons and their actions," *Ann. Rev. Biochem. 56*, 727–777.

Prouty et al., 1975, "Degradation of abmormal proteins in *Escherichia coli*: Formation of protein inclusion in cells exposed to amino acid analogs," *The Journal of Biological Chemistry 250*(3):1112–1129.

Rubinstein et al., 1979, "Human leukocyte interferon: production, purification to homogeneity, and initial characterization," *Proc. Natl. Acad. Sci. U.S.A. 76*(2):640–644.

Rubinstein et al., 1981, "Human Leukocye Interferon: Isolation and Characterization of Several Molecular Forms," *Arch. Biochem. Biophys. 210*(1):307–318.

(List continued on next page.)

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(57) **ABSTRACT**

Disclosed herein are methods and means of microbially producing, via recombinant DNA technology, mature human leukocyte interferons, useful in the treatment of viral and neoplastic diseases.

22 Claims, 19 Drawing Sheets

OTHER PUBLICATIONS

Staehelin et al., 1981, "Production of hybridomas secreting monoclonal antibodies to the human leukocyte interferons," *Proc. Natl. Acad. Sci. U.S.A. 78*(3):1848–1852.
Stebbing et al., "Biological Comparison of Natural and Recombinant DNA–Derived Polypeptides," *State of the Art: Insulins and Growth Hormone*. Presentation for FDA Meeting, Jun. 3–4, 1980.
Streuli et al., 1980, "At least three human type α interferons: Structure of α2," *Science 209*:1343–1347.
Torma and Paucker, 1976, "Purification and characterization of human leukocyte interferon components," *J. Biol. Chem. 251*(16):4810–4816.
Transcript of Tape Recorded Press Conference in Boston, Mass. at the Park Plaza Hotel, Jan. 16, 1980.
Zoon et al., 1979, "Purification and partial characterization of human lymphoblast interferon," *Proc. Natl. Acad. Sci. U.S.A. 76*(11):5601–5605.
Backman et al., Cell 13:65–71 (1978).
Chang et al., Nature 275:617–624 (1978).
Goeddel et al., Nature 281:544–548 (1979).
Taniguchi et al., Proc. Nat'l. Acad. Sci. USA 77:5230–5233 (published Oct. 15, 1980).
Guarente et al., Science 209:1428–1430 (published Sep. 19, 1980).
Derynck et al., Nature 287:193–197 (published Sep. 18, 1980).
Houghton, Nature 285:536 (published Jun. 19, 1980).
Derynck et al., Nature 285:542–547 (published Jun. 19, 1980).
Taniguchi et al., Nature 285:547–549 (published Jun. 19, 1980).
Goeddel et al., Nature 287:411–416 (published Oct. 2, 1980).
Guarente et al., Cell 20:543–553 (published Jun. 20 or 21, 1980).
Roberts et al., Proc. Nat'l. Acad. Sci. USA 76:5596–5600 (1979).
Nagata et al., 287:401–408 (1980).
Mantei et al., Gene 10:1–10 (1980).
Zoon et al., Science 207:527–528 (Feb. 1, 1980).
Zoon et al., Ann. N.Y. Acad. Sci. 350:390–398 (Oct. 1980).
Anonymous, Research Disclosure (Jul. 1979).
Weissmann, Interferon 1981, 3:101–134 (1981).
Nagata et al; Nature 284: 316 (1980).*
Roberts et al; Proc. Natl. Acad. Sci. USA 76: 760 (1979).*

* cited by examiner

		TRYPTIC PEPTIDE (T-1)	TRYPTIC PEPTIDE (T-13)
PROTEIN		Ala-Glu-Ile-Met-Arg	---His-Glu-Met-Ile-Gln---
mRNA		5' GCN GA$^{A}_{G}$ AU$^{A}_{C}$ AUG A$^{A}_{U}$GN	5' CA$^{C}_{U}$ GA$^{A}_{G}$ AUG AU$^{A}_{U}$ CA$^{A}_{G}$
COMPLEMENTARY DNA PRIMERS	3'	CTT TA$^{A}_{G}$^{T} TAC GC (T-1A)	3' GTA CTT TAC TA (T-13A)
		--C---------- (T-1B)	--G---------- (T-13B)
		----------T- (T-1C)	------C------- (T-13C)
		--C---------T- (T-1D)	--G---C------- (T-13D)

FIG.1

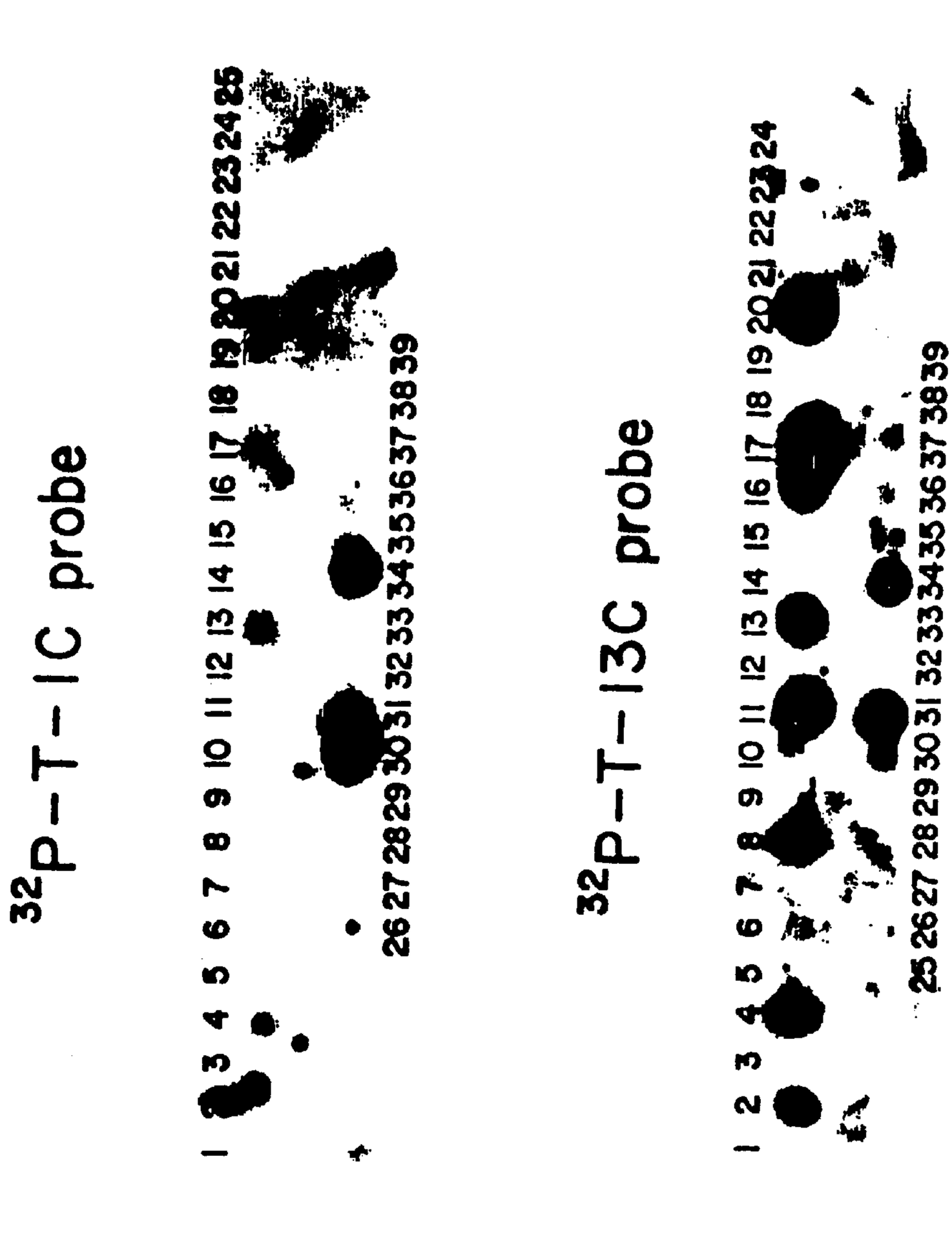
32P-T-1C probe
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24 25 26 27 28 29 30 31 32 33 34 35 36 37 38 39
32P-T-13C probe
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24 25 26 27 28 29 30 31 32 33 34 35 36 37 38 39

FIG. 2

```
                -60                 -40                 -20                  +1                  20                  40
                 |                   |                   |                   |                   |                   |
LeIF A TGAGCCTAAACCTTAGGCTCACCCATTTCAACCAGTCTAGCAGCATCTGCAACATCTACAATGGCCTTGACCTTTGCTTTACTGGTGGCCCTCCTGGTGC
LeIF B                                                  TACTAGCTCAGCAGCATCCGCAACATCTACAATGGCCTTGACTTTTTATTTAATGGTGGCCCTAGTGGTGC
LeIF C                        CAAGGTTATCCATCTCAAGTAGCCTAGCAATATTTGCAACATCCCAATGGCCCTGTCCTTTTCTTTACTTATGGCCGTGCTGGTGC
LeIF D              CAAGGTTCAGAGTCACCCATCTCAGCAAGCCCAGAAGTATCTGCAATATCTACGATGGCCTCGCCCTTTGCTTTACTGATGGTCCTGGTGGTGC
LeIF E
LeIF F                                                                  ACATCCCAATGGCCCTGTCCTTTTCTTTACTGATGGCCGTGCTGGTGC
LeIF G
LeIF H            CCAAGGTTCAGTGTTACCCCTCATCAACCAGCCCAGCAGCATCTTCGGGATTCCCAATGGCATTGCCCTTTGCTTTAATGATGGCCCTGGTGGTGC

                          60                  80                 100                 120                 140
                           |                   |                   |                   |                   |
LeIF A TCAGCTGCAAGTCAAGCTGCTCTGTGGGCTGTGATCTGCCTCAAACCCACAGCCTGGGTAGCAGGAGGACCTTGATGCTCCTGGCACAGATGAGGAAAAT
LeIF B TCAGCTACAAGTCATTCAGCTCTCTGGGCTGTGATCTGCCTCAGACTCACAGCCTGGGTAACAGGAGGGCCTTGATACTCCTGGCACAAATGCGAAGAAT
LeIF C TCAGCTACAAATCCATCTGTTCTCTGGGCTGTGATCTGCCTCAGACCCACAGCCTCGGTAATAGGAGGGCCTTGATACTCCTGGGACAAATGGGAAGAAT
LeIF D TCAGCTGCAAGTCAAGCTGCTCTCTGGGCTGTGATCTCCCTGAGACCCACAGCCTGGATAACAGGAGGACCTTGATGCTCCTGGCACAAATGAGCAGAAT
LeIF E                     CTGCCTCTGGGCTGTGATCTGCCTCAGGCCCACAGCGTGGGTAACAGGAGGGCCTTCATACTCCTGACACAAATGAGGAGAAT
LeIF F TCAGCTACAAATCCATCTGTTCTCTGGGCTGTGATCTGCCTCAGACCCACAGCCTGGGTAATAGGAGGGCCTTGATACTCCTGGCACAAATGGGAAGAAT
LeIF G
LeIF H TCAGCTGCAAGTCAAGCTGCTCTCTGGGCTGTAATCTGTCTCAAACCCACAGCCTGAATAACAGGAGGACTTTGATGCTCATGGCACAAATGAGGAGAAT
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FIG.3A

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                    160       180       200       220       240
                    |         |         |         |         |
LeIF A CTCTCTTTTCTCCTGCTTGAAGGACAGACATGACTTTGGATTTCCC CAGGAGGAGTTT   GGCAACCAGTTCCAAAAGGCTGAAACCATCCCTGTCCT
LeIF B CTCTCCTTTCTCCTGCCTGAAGGACAGACATGACTTTGAATTCCCC CAGGAGGAGTTTGATGATAAACAGTTCCAGAAGGCTCAAGCCATCTCTGTCCT
LeIF C CTCTCCTTTCTCCTGCCTGAAGGACAGACATGATTTCCGAATCCCC CAGGAGGAGTTTGATGGCAACCAGTTCCAGAAGGCTCAAGCCATCTCTGTCCT
LeIF D CTCTCCTTCCTCCTGTCTGATGGACAGACATGACTTTGGATTTCCC CAGGAGGAGTTTGATGGCAACCAGTTCCAGAAGGCTCCAGCCATCTCTGTCCT
LeIF E CTCTCCTTTTTCTTACCTGAAGGACAGACATGACTTTGATTTTCCATCATCAGGTGTTTCATGGCAACCACTTCCAGAAGGTTCAAGCTATCTTCCTTTT
LeIF F CTCTCCTTTCTCCTGCCTGAAGGACAGACATGACTTTGGATTCCCC CAAGAGGAGTTTGATGGCAACCAGTTCCAGAAGGCTCAAGCCATCTCTGTCCT
LeIF G                          CATGACTTTGGATTTCCT CAGGAGGAGTTTGATGGCAACCAGTTCCAGAAGGCTCAAGCCATCTCTGTCCT
LeIF H CTCTCCTTTCTCCTGCCTGAAGGACAGACATGACTTTGAATTTCCC CAGGAGGAATTTGATGGCAACCAGTTCCAGAAAGCTCAAGCCATCTCTGTCCT

                    260       280       300       320       340
                    |         |         |         |         |
LeIF A CCATGAGATGATCCAGCAGATCTTCAATCTCTTCAGCACAAAGGACTCATCTGCTGCTTGGGATGAGACCCTCCTAGACAAATTCTACACTGAACTCTAC
LeIF B CCATGAGATGATCCAGCAGACCTTCAACCTCTTCAGCACAAAGGACTCATCTGCTGCTTTGGATGAGACCCTTCTAGATGAATTCTACATCGAACTTGAC
LeIF C CCATGAGATGATCCAGCAGACCTTCAATCTCTTCAGCACAGAGGACTCATCTGCTGCTTGGGAACAGAGCCTCCTAGAAAAATTTTCCACTGAACTTTAC
LeIF D CCATGAGCTGATCCAGCAGATCTTCAACCTCTTTACCACAAAAGATTCATCTGCTGCTTGGGATGAGGACCTCCTAGACAAATTCTGCACCGAACTCTAC
LeIF E CCATGAGATGATGCAGCAGACCTTCAACCTCTTCAGCACAAAGGACTCATCTGATACTTGGGATGAGACCCTTTTAGACAAATCCTACACTGAACTTTAC
LeIF F CCATGAGATGATCCAGCAGACCTTCAATCTCTTCAGCACAAAGGACTCATCTGCTACTTGGGAACAGAGCCTCCTAGAAAAATTTTCCACTGAACTTAAC
LeIF G CCATGAGATGATCCAGCAGACCTTCAATCTCTTCAGCACAAAGGACTCATCTGCTACTTGGGATGAGACACTTCTAGACAAATTCTACACTGAACTTTAC
LeIF H CCATGAGATGATGCAGCAGACCTTCAATCTCTTCAGCACAAAGAACTCATCTGCTGCTTGGGATGAGACCCTCCTAGAAAAATTCTACATTGAACTTTTC
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FIG.3B

```
                    360        380        400        420        440
                    |          |          |          |          |
LeIF A CAGCAGCTGAATGACCTGGAAGCCTGTGTGATACAGGGGGTGGGGGTGACAGAGACTCCCCTGATGAAGGAGGACTCCATTCTGGCTGTGAGGAAATACT
LeIF B CAGCAGCTGAATGACCTGGAAGTCCTGTGTGATCAGGAAGTGGGGGTGATAGAGTCTCCCCTGATGTACGAGGACTCCATCCTGGCTGTGAGGAAATACT
LeIF C CAGCAACTGAATGACCTGGAAGCATGTGTGATACAGGAGGTTGGCGTGGAAGAGACTCCCCTGATGAATGAGGACTCCATCCTGGCTGTGAGGAAATACT
LeIF D CAGCAGCTGAATGACTTGGAAGCCTGTGTGATGCAGGAGGAGAGGGTGGGAGAAACTCCCCTGATGAATGTGGACTCCATCTTGGCTGTGAAGAAATACT
LeIF E CAGCAGCTGAATGACCTGGAAGCCTGTGTGATGTAGAAGGTTGGAGTGGAAGAGACTCCCCTGAGGAATGTGGACTCCATCCTGGCTGTGAGAAAATACT
LeIF F CAGCAGCTGAATGACATGGAAGCCTGCGTGATACAGGAGGTTGGGGTGGAAGAGACTCCCCTGATGAATGTGGACTCCATCTTGGCTGTGAAGAAATACT
LeIF G CAGCAGCTGAATGACCTGGAAGCCTGTATGATGCAGGAGGTTGGAGTGGAAGACACTCCTCTGATGAATGTGGACTCTATCCTGACTGTGAGAAAATACT
LeIF H CAGCAAATGAATGACCTGGAAGCCTGTGTGATACAGGAGGTTGGGGTGGAAGAGACTCCCCTGATGAATGAGGACTCCATCCTGGCTGTGAAGAAATACT

                    460        480        500        520        540
                    |          |          |          |          |
LeIF A TCCAAAGAATCACTCTCTATCTGAAAGAGAAGAAATACAGCCCTTGTGCCTGGGAGGTTGTCAGAGCAGAAATCATGAGATCTTTTTCTTTGTCAACAAA
LeIF B TCCAAAGAATCACTCTATATCTGACAGAGAAGAAATACAGCTCTTGTGCCTGGGAGGTTGTCAGAGCAGAAATCATGAGATCCTTCTCTTTATCAATCAA
LeIF C TCCAAAGAATCACTCTTTATCTAATAGAGAGGAAATACAGCCCTTGTGCCTGGGAGGTTGTCAGAGCAGAAATCATGAGATCCCTCTCGTTTTCAACAAA
LeIF D TCCGAAGAATCACTCTCTATCTGACAGAGAAGAAATACAGCCCTTGTGCCTGGGAGGTTGTCAGAGCAGAAATCATGAGATCCCTCTCTTTATCAACAAA
LeIF E TTCAAAGAATCACTCTTTATCTGACAAAGAAGAAGTATAGCCCTTGTTCCTGGGAGGCTGTCAGAGCAGAAATCATGAGATCCTTCTCTTTATGAACGAA
LeIF F TCCAAAGAATCACTCTTTATCTGACAGAGAAGAAATACAGCCCTTGTGCTTGGGAGGTTGTCAGAGCAGAAATCATGAGATCCTTCTCTTTATCAAAAAT
LeIF G TTCAAAGAATCACCCTCTATCTGACAGAGAAGAAATACAGCCCTTGTGCATGGGAGGTTGTCAGAGCAGAAATCATGAGATCCTTCTCTTTATCAGCAAA
LeIF H TCCAAAGAATCACTCTTTATCTGATGGAGAAGAAATACAGCCCTTGTGCCTGGGAGGTTGTCAGAGCAGAAATCATGAGATCCTTCTCTTTTTCAACAAA
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FIG.3C

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                560        580        600        620        640
                |          |          |          |          |          |          |          |          |
LeIF A CTTGCAAGAAAGTTTAAGAAGTAAGGAATGAAAACTGGTTCAACATGGAAATGATTTTCATTGATTCGTATGCCAGCTCACCTTTTTATGATCTGCCATT
LeIF B CTTGCAAAAAAAGATTGAAGAGTAAGGAATGAGACCTGGTACAACACGGAAATGATTCTCATAGACTAATACAGCAGTCTACACTTTGACAAGTTGTGCTC
LeIF C CTTGCAAAAAAAGATTAAGGAGGAAGGATTGAAAACTGGTTCAACATGGCAATGATCCTGATTGACTAATACATTATCTCACACTTTCATGAGTTCTTCCA
LeIF D CTTGCAAGAAAGATTAAGGAGGAAGGAATAATATCTGGTCCAACATGAAAACAATTCTTATTGACTCATACACCAGGTCACGCTTTCATGAATTCTGTCA
LeIF E CTTGCAGGAAAGATTAAGGAGGAAGGAATGAAAACTGGTTCAACATGGAAATGAGAAACATTTCCATGATTAATACATCATCTCACACATTCATGAATTC
LeIF F TTTTCAAGAAAGATTAAGGAGGAAGGAATGAAACCGTTTCAACATGGAAATGATCTGTATTGACTAATACACCAGTCCACACTTCTATGACTTCTGCCAT
LeIF G CTTGCAAGAAAGATTAAGGAGGAAGGAATGAAAACTGGTTCAACATCGAAATGATTCTCATTGACTAGTACACCATTTCACACTTCTTGAGTTCTGCCGT
LeIF H CTTGCAAAAAAGATTAAGGAGGAAGGATTGAAAACTGGTTCATCATGGAAATGATTCTCATTGACTAATACATCATCTCACACTTTCATGTTCTTCCATT

                660        680        700        720        740
                |          |          |          |          |          |          |          |          |
LeIF A TCAAAGACTCATGTTTCTGCTATGACCATGACACGATTTAAATCTTTTCAAATGTTTTTAGGAGTATTAATCAACATTGTATTCAGCTCTTAAGGCACTA
LeIF B TTTCAAAGACCCTTGTTTCTGCCAAAACCATGCTATGAATTGAATCAAATGTGTCAAGTGTTTTCAGGAGTGTTAAGCAACATCCTGTTCAGCTGTATGG
LeIF C TTTCAAAGACTCACTTCTATAACCACGACGCGTTGAATCAAAATTTTCAAATGTTTTCAGCAGTGTAAAGAAGTGTCGTGTATACCTGTGCAGGCACTAG
LeIF D TTTCAAAGACTCTCACCCCTGCTATAACTATGACCATGCTGATAAACTGATTTATCTATTTAAATATTTATTTAACTATTCATAAGATTTAAATTATTTT
LeIF E TGCCATTTCTCATTTTTGCTATATCCATGACATGAGTTGAATCAAAATTTTAAAATGTTTTCAGGAATGTTAAGCAGCATCATGTTCAGCTGTACAGGCA
LeIF F TTCAAAGACTCATTTCTCCTATAACCACCGCATGAGTTGAATCAAAATTTTCAGATCTTTTCAGGAGTGTAAGGAAACATCATGTTTACCTGTGCAGGCA
LeIF G TTCAAATATTAATTTCTGCTATATCCATGACTTGAGTTGAATCAAAATTTTCAAACGTTTCACACGTGTTAAGCAACACTTCTTTAGCTCCACAGGGACA
LeIF H TCAAAGACTCACTTCTATAACCACCACAAGTTGAATCAAAATTTCCAAATGTTTTCAGGAGTGTTAAGAAGCATCGTGTTTACCTGTGCAGGCACTAGTC
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FIG.3D

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                    760                 780                 800                 820                 840
                    |                   |                   |                   |                   |
LeIF A GTCCCTTACAGAGGACCATGCTGACTGATCCATTATCTATTTAAATATTTTTAAAATATTATTTATTTAACTATTTATAAAACAACTTATTTTTGTTCAT
LeIF B GCACTAGTCCCTTACAGATGACCATGCTGATGGATCTATTCATCTATTTATTTAAATCTTTATTTAGTTAACTACTATAGGGACTTAAATTAGTTTTGTT
LeIF C TCCTTTACAGATGACCATTCTGATGTCTCTGTTCATCTTTTGTTTAAATATTTATTTAATTATTTTTAAAATTTATGTAATATCATGAGTCGCTTTACAT
LeIF D TGTTCATATAACGTCATGTGCACCTTTACACTGTGGTTAGTGTAATAAAACATGTTCCTTATATTTACTC-poly(A)
LeIF E CTAGTTCCTTACGGATGATCATGCTGATGGATCTGTTTATCTATTTGTCTAAATAATTATTTAACTATTTATAATATTTAAAATCTTCTTTTCATGTATC
LeIF F CTAGTCCTTTACAGATGACCATGCTGATAGATCTAATTATCTATCTATTGAAATATTTATTTATTTATTAGATTTAAATTATTTTTGTCCATGTAATATT
LeIF G AAATCTTTACAGATGATCATGCCAATCTATCTATTCTATCTATTTATCTATCTGTCTGTCTTCTATCTAATCTATTTAAATATTTATTTATTTATAAGAT
LeIF H CTTTACAGATGACCATTCTGATGTCTCCTTTCATCTATTTATTTAAATATTTATTTATTTAACTATTTTTATTATTTAAATTATTTTTTATGTAATATCA

                    860                 880                 900                 920                 940
                    |                   |                   |                   |                   |
LeIF A ATTACGTCATGTGCACCTTTGCACAGTGGTTAATGTAATAAAATATGTTCTTTGTATTTGGT-poly(A)
LeIF B CATATTATATTATGTGAACTTTTACATTGTGAATTGTGTAACAAAAACATGTTCTTATATTTATTATTTTGCCATGTTTATTAAATTTTTACTATGAAAA
LeIF C TGTGGTTAATGTAACAATATATGTTCTTCATATTTAGCCAATATATTAATTTCCTTTTTCATTAAATTTTTACTATAC-poly(A)
LeIF E ATGTATTTTTACTTTGTGGTTAATATAACAACACATGTTCTTTATATTTAGTCAATATATTACTTTGCTTTTTTCATTAAATTTTTACTATGG-poly(A)
LeIF F ATGTGTACTTTTACATTGTGTTATATCAAAATATGTTAATCTAATATTTAGTCAATATATTATTTTCTTTTTATTAATTTTTACTATTAAAACTTCTTAT
LeIF G TTAAATTATTTTAAACTTATGTTTGTTCAGGTAATATTACATCCACCTTACTTTGTGGCTAATATAATAAAATATGTTCTTTATGTTTTGTC-poly(A)
LeIF H TGAGTACCTTTACATTGTGGTTAATGTAACAAATATGTTCTTCATATTTAGCCAATATATTAATTTCCTTTTTCATTAAATTTTTACTAT-poly(A)

                960                 980                 1000
                |                   |                   |
LeIF B AATTCTTTATTTATTCTTTAAAATTGAACTCCAACCCATGAATTGTGCAAACTGATTAAAGAATGGATGGT-poly(A)
LeIF F ATTATTTGGTTATTCTTTAATAAAGAAATTCCAAGCCC-poly(A)
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FIG.3E

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          S1       S10        S20 S23        10         20         30         40
          |        |          |   |          |          |          |          |
LeIF A    MALTFALLVALLVLSCKSSCSVGCDLPQTHSLGSRRTLMLLAQMRKISLFSCLKDRHDFGFPQ
LeIF B    MALTFYLMVALVVLSYKSFSSLGCDLPQTHSLGNRRALILLAQMRRISPFSCLKDRHDFEFPQ
LeIF C    MALSFSLLMAVLVLSYKSICSLGCDLPQTHSLGNRRALILLGQMGRISPFSCLKDRHDFRIPQ
LeIF D    MASPFALLMVLVVLSCKSSCSLGCDLPETHSLDNRRTLMLLAQMSRISPSSCLMDRHDFGFPQ
LeIF E                              LPLGCDLPQAHSVGNRRAFILLTQMRRISPFSYLKDRHDFDFPH
LeIF F    MALSFSLLMAVLVLSYKSICSLGCDLPQTHSLGNRRALILLAQMGRISPFSCLKDRHDFGFPQ
LeIF G                                                                HDFGFPQ
LeIF H    MALPFSLMMALVVLSCKSSCSLGCNLSQTHSLNNRRTLMLMAQMRRISPFSCLKDRHDFEFPQ

All       MA  F L    VLS KS  S GC L  THSL  RR L L  QM  IS  SCL DRHDF  PQ
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```
                    50         60         70         80         90         100
                    |          |          |          |          |          |
LeIF A    EEF-GNQFQKAETIPVLHEMIQQIFNLFSTKDSSAAWDETLLDKFYTELYQQLNDLEACVIQG
LeIF B    EEFDDKQFQKAQAISVLHEMIQQTFNLFSTKDSSAALDETLLDEFYIELDQQLNDLEVLCDQE
LeIF C    EEFDGNQFQKAQAISVLHEMIQQTFNLFSTEDSSAAWEQSLLEKFSTELYQQLNDLEACVIQE
LeIF D    EEFDGNQFQKAPAISVLHELIQQIFNLFTTKDSSAAWDEDLLDKFCTELYQQLNDLEACVMQE
LeIF E    QVFHGNHFQKVQAIFLFHEMMQQTFNLFSTKDSSDTWDETLLDKSYTELYQQLNDLEACVM*K
LeIF F    EEFDGNQFQKAQAISVLHEMIQQTFNLFSTKDSSATWEQSLLEKFSTELNQQLNDMEACVIQE
LeIF G    EEFDGNQFQKAQAISVLHEMIQQTFNLFSTKDSSATWDETLLDKFYTELYQQLNDLEACMMQE
LeIF H    EEFDGNQFQKAQAISVLHEMMQQTFNLFSTKNSSAAWDETLLEKFYIELFQQMNDLEACVIQE

All       EEFD  QFQKA  I VLHE  QQ FNLF T  SSA     LL  F  EL QQ ND E    Q
```

FIG.4A

```
                  110       120       130       140       150       160   166
                   |         |         |         |         |         |    |
LeIF A   VGVTETPLMKEDSILAVRKYFQRITLYLKEKKYSPCAWEVVRAEIMRSFSLSTNLQESLRSKE
LeIF B   VGVIESPLMYEDSILAVRKYFQRITLYLTEKKYSSCAWEVVRAEIMRSFSLSINLQKRLKSKE
LeIF C   VGVEETPLMNEDSILAVRKYFQRITLYLIERKYSPCAWEVVRAEIMRSLSFSTNLQKRLRRKD
LeIF D   ERVGETPLMNVDSILAVKKYFRRITLYLTEKKYSPCAWEVVRAEIMRSLSLSTNLQERLRRKE
LeIF E   VGVEETPLRNVDSILAVRKYFQRITLYLTKKKYSPCSWEAVRAEIMRSFSL*TNLQERLRRKE
LeIF F   VGVEETPLMNVDSILAVKKYFQRITLYLTEKKYSPCAWEVVRAEIMRSFSLSKIFQERLRRKE
LeIF G   VGVEDTPLMNVDSILTVRKYFQRITLYLTEKKYSPCAWEVVRAEIMRSFSLSANLQERLRRKE
LeIF H   VGVEETPLMNEDSILAVRKYFQRITLYLMEKKYSPCAWEVVRAEIMRSFSFSTNLQKRLRRKD
All      V     PLM  DSIL V KYF RITLYL E KYS CAWEVVRAEIMRS S S   Q  L   K
```

FIG.4B

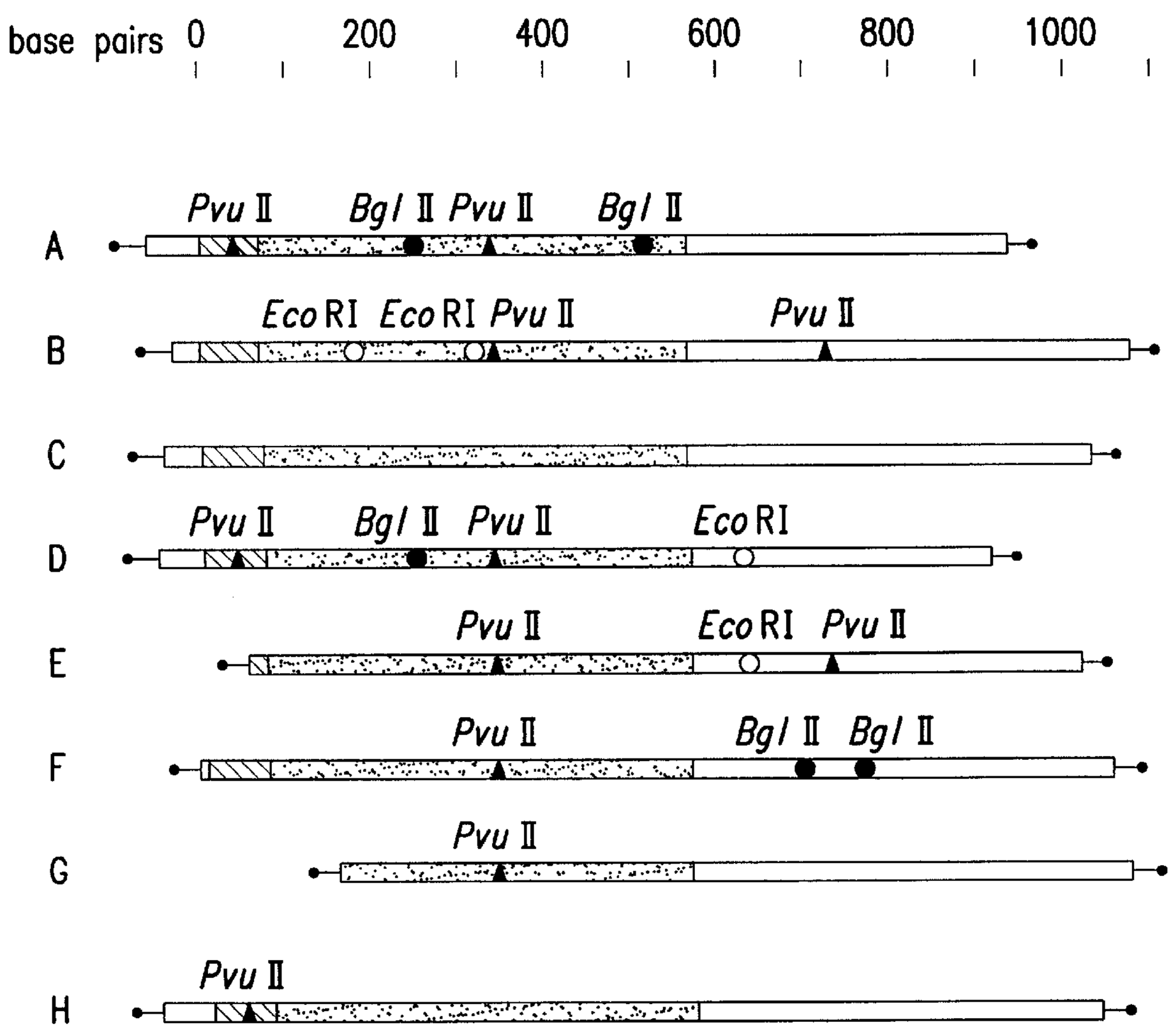

base pairs 0
200
400
600
800
1000
A
Pvu II
Bgl II
Pvu II
Bgl II
B
EcoRI
EcoRI
Pvu II
Pvu II
C
D
Pvu II
Bgl II
Pvu II
EcoRI
E
Pvu II
EcoRI
Pvu II
F
Pvu II
Bgl II
Bgl II
G
Pvu II
H
Pvu II

FIG.5

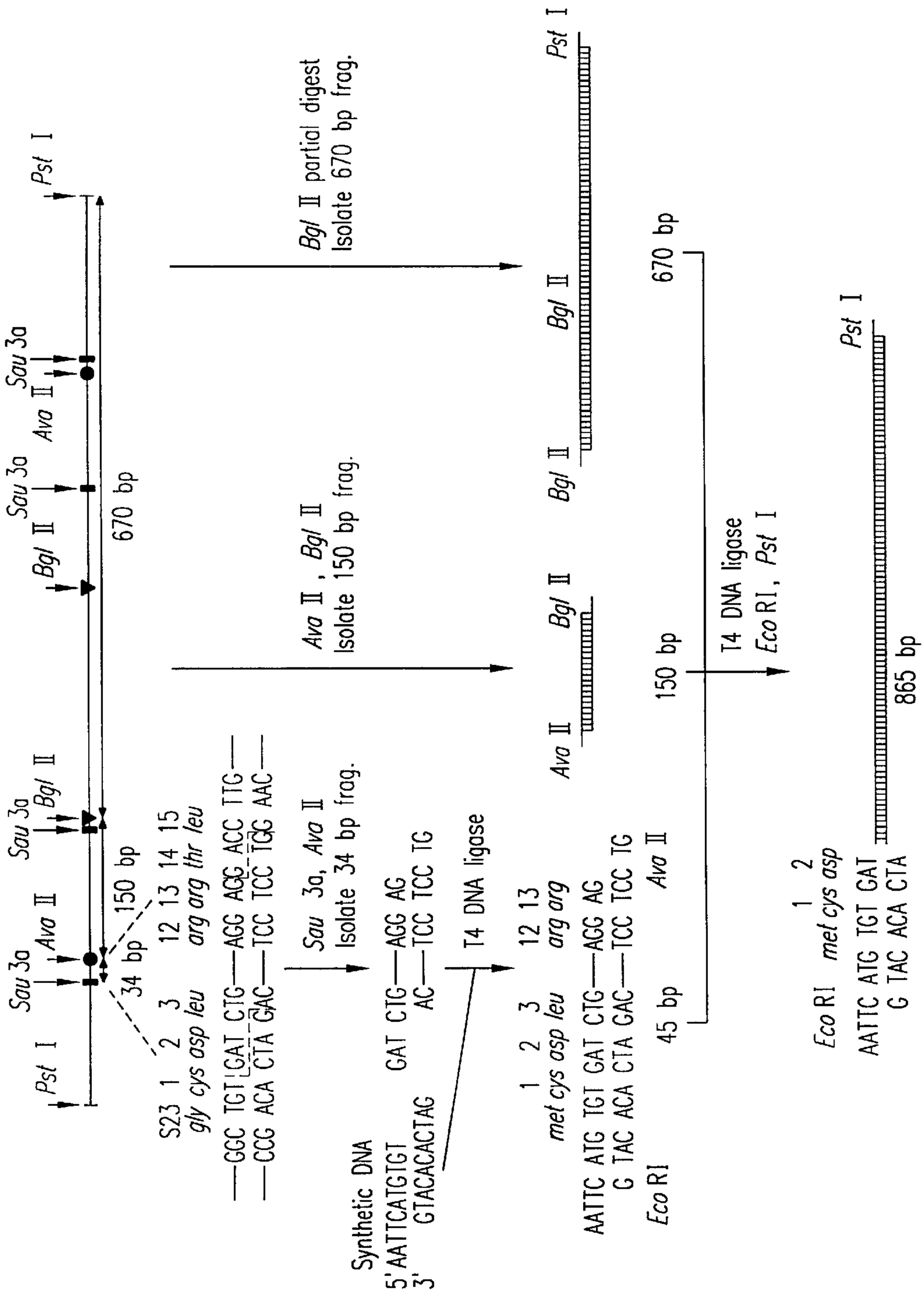

Pst I
Sau 3a
Bgl II
Ava II
Sau 3a
Pst I
670 bp
Bgl II partial digest
Isolate 670 bp frag.
Ava II , Bgl II
Isolate 150 bp frag.
Pst I
Bgl II
Bgl II
Bgl II
670 bp
150 bp
T4 DNA ligase
Eco RI , Pst I
Pst I
865 bp
LeIF A
Sau 3a
Ava II
Sau 3a
Bgl II
Pst I
34 bp
150 bp
S23 1 2 3 12 13 14 15
gly cys asp leu arg arg thr leu
—GGC TGT GAT CTG—AGG AGG ACC TTC—
—CCG ACA CTA GAC—TCC TCC TGG AAC—
Sau 3a, Ava II
Isolate 34 bp frag.
Synthetic DNA
5'AATTCATGTGT GAT CTG—AGG AG
3' GTACACACTAG AC—TCC TCC TG
T4 DNA ligase
1 2 3 12 13
met cys asp leu arg arg
AATTC ATG TGT GAT CTG—AGG AG
G TAC ACA CTA GAC—TCC TCC TG
Eco RI
45 bp
Ava II
Ava II
1 2
Eco RI met cys asp
AATTC ATG TGT GAT
G TAC ACA CTA

FIG.6

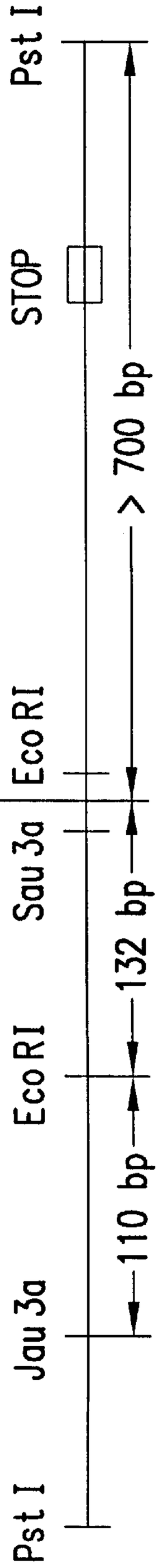

Pst I
STOP
> 700 bp
Xba
Eco RI
Sau 3a
132 bp
Eco RI
Sau 3a
110 bp
GATCTG
GGCTGT
Pst I

FIG. 7a

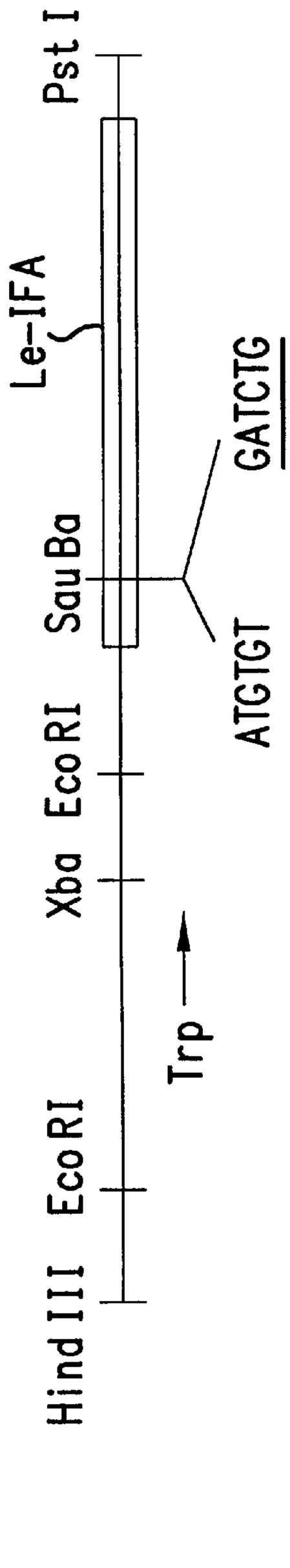

Pst I
Le-IFA
GATCTG
Sau Ba
ATGTGT
Eco RI
Xba
Eco RI
Trp
Hind III

FIG. 7b

```
A                                                                                                                    GTATGTTCCCTA
H                                                                                                                    GTATGTTCCCTA
I                                                                                                                    GTATGTTCCTTA
J                                                                                                                    GTATGTTCACTA
C                                                                                                                    GTATGTTCACTA

A    TTTAAGGC-TAGGCACAAAGCAAGGTCTTCAGAGAACCTGGAGCCTAAGGTTTAGGCTCACCCATT-TCAACCAGTCTAGCAGCATCTGCAACATCTACA
H    TTTAAGGC-TAGGCACAAAGCAAGGTCTTCAGAGAACCTGGAGCCTAAGGTTTAGGCTCACCCATT-TCAACCAGTCTAGCAGCATCTGCAACATCTACA
I    TTTAAGACCTATGCACAGAGCAAGGTCTTCAGAAAACCTACAACCCAAGGTTCAGTGTTACCCCTCATCAACCAGCCCAGCAGCATCTTCAGGGTTCCCA
J    TTTAAGGCCTATGCACAGAGCAAAGTCTTCAGAAAACCTAGAGGCCAAAGTTCAAGGTTACCCATC-TCAAGTAGCCTAGCAACATTTGCAACATCCCA-
C    TTTAAGACCTATGCACAGAGCAAAGTCTCCAGAAAACCTAGAGGCCACGGTTCAA-GTTACCCACC-TCAGGTAGCCTAGTGATATTTGCAAAATCCCA-

     +1                                                                                                              100
A    ATGGCCTTGACCTTTGCTTTACTGGTGGCCCTCCTGGTGCTCAGCTGCAAGTCAAGCTGCTCTGTGGGCTGTGATCTGCCTCAAACCCACAGCCTGGGTA
H    ATGGCATTGCCCTTTGCTTTAATGATGGCCCTGGTGGTGCTCAGCTGCAAGTCAAGCTGCTCTCTGGGCTGTAATCTGTCTCAAACCCACAGCCTGAATA
I    ATGGCCCTGTCCTTTTCTTTACTGATGGCCGTGCTGGTGCTCAGCTACAAATCCATCTGTTCTCTAGGCTGTGATCTGCCTCAGACCCACAGCCTGGGTA
J    ATGGCCCGGTCCTTTTCTTTACTGATGGTCGTGCTGGTACTCAGCTACAAATCCATCTGCTCTCTGGGCTGTGATCTGCCTCAGACCCACAGCCTGCGTA
C    ATGGCCCTGTCCTTTTCTTTACTTATGGCCGTGCTGGTGCTCAGCTACAAATCCATCTGATCTCTGGGCTGTGATCTGCCTCAGACCCACACCCTGCGTA

                                                                                                                     200
A    GCAGGAGGACCTTGATGCTCCTGGCACAGATGAGGAGAATCTCTCTTTTCTCCTGCTTGAAGGACAGACATGACTTTGGATTTCCCCAGGAGGAGTTT--
H    ACAGGAGGACTTTGATGCTCATGGCACAAATGAGGAGAATCTCTCCTTTCTCCTGCCTGAAGGACAGACATGACTTTGAATTTCCCCAGGAGGAATTTGA
I    ATAGGAGGGCCTTGATACTCCTGGCACAAATGGGAAGAATCTCTCCTTTCTCCTGCCTGAAGGACAGACCTGACTTTGGACTTCCCCAGGAGGAGTTTGA
J    ATAGGAGGGCCTTGATACTCCTGGCACAAATGGGAAGAATCTCTCCTTTCTCCTGCTTGAAGGACAGACATGAATTCAGATTCCCAGAGGAGGAGTTTGA
C    ATAGGAGGGCCTTGATACTCCTGGGACAAATGGGAAGAATCTCTCCTTTCTCCTGCCTGAAGGACAGACATGATTTCCGAATCCCCCAGGAGGAGTTTGA
```

FIG.8A

```
                                                                                                300
A     -GGCAACCAGTTCCAAAAGGCTGAAACCATCCCTGTCCTCCATGAGATGATCCAGCAGATCTTCAATCTCTTCAGCACAAAGGACTCATCTGCTGCTTGG
H     TGGCAACCAGTTCCAGAAAGCTCAAGCCATCTCTGTCCTCCATGAGATGATGCAGCAGACCTTCAATCTCTTCAGCACAAAGAACTCATCTGCTGCTTGG
I     TGGCAACCAGTTCCAGAAGACTCAAGCCATCTCTGTCCTCCATGAGATGATCCAGCAGACCTTCAATCTCTTCAGCACAGAGGACTCATCTGCTGCTTGG
J     TGGCCACCAGTTCCAGAAGACTCAAGCCATCTCTGTCCTCCATGAGATGATCCAGCAGACCTTCAATCTCTTCAGCACAGAGGACTCATCTGCTGCTTGG
C     TGGCAACCAGTTCCAGAAGGCTCAAGCCATCTCTGTCCTCCATGAGATGATCCAGCAGACCTTCAATCTCTTCAGCACAGAGGACTCATCTGCTGCTTGG

                                                                                                400
A     GATGAGACCCTCCTAGACAAATTCTACACTGAACTCTACCAGCAGCTGAATGACCTGGAAGCCTGTGTGATACAGGGGGTGGGGGTGACAGAGACTCCCC
H     GATGAGACCCTCCTAGAAAAATTCTACATTGAACTTTTCCAGCAAATGAATGACCTGGAAGCCTGTGTGATACAGGAGGTTGGGGTGGAAGAGACTCCCC
I     GAACAGAGCCTCCTAGAAAAATTTTCCACTGAACTTTACCAGCAACTGAATAACCTGGAAGCATGTGTGATACAGGAGGTTGGGGATGGAAGAGACTCCCC
J     GAACAGAGCCTCCTAGAAAAATTTTCCACTGAACTTTACCAGCAACTGAATGACCTGGAAGCATGTGTGATACAGGAGGTTGGGGTGGAAGAGACTCCCC
C     GAACAGAGCCTCCTACAAAAATTTTCCACTGAAATTTACCAGCAACTGAATGACCTGGAAGCATGTGTGATACAGGAGGTTGGGGTGGAAGAGACTCCCC

                                                                                                500
A     TGATGAAGGAGGACTCCATTCTGGCTGTGAGGAAATACTTCCAAAGAATCACTCTCTATCTGAAAGAGAAGAAATACAGCCCTTGTGCCTGGGAGGTTGT
H     TGATGAATGAGGACTCCATCCTGGCTGTGAAGAAATACTTCCAAAGAATCACTCTTTATCTGATGGAGAAGAAATACAGCCCTTGTGCCTGGGAGGTTGT
I     TGATGAATGAGGACTCCATCCTGGCTGTGAGGAAATACTTCCAAAGAATCACTCTTTATCTAACAGAGAAGAAATACAGCCCTTCAGCCTGGGAGGTTGT
J     TGATGAATGAGGACTTCATCCTGGCTGTGAGGAAATACTTCCAAAGAATCACTCTTTATCTAATGGAGAAGAAATACAGCCCTTGTGCCTGGGAGGTTGT
C     TGATGAATGAGGACTCCATCCTGGCTGTGAGGAAATACTTCCAAAGAATCACTCTTTATCTAATAGAGAGGAAATACAGCCCTTGTGCCTGGGAGGTTGT
```

FIG.8B

```
                                                                                                    600
A    CAGAGCAGAAATCATGAGATCTTTTTCTTTGTCAACAAACTTGCAAGAAAGTTTAAGAAGTAAGGAATGAAAACTGGTTCAACATGGAAATGATTTTCAT
H    CAGAGCAGAAATCATGAGATCCCTCTCTTTTTCAACAAACTTGCAAAAAAGATTAAGGAGGAAGGATTGAAAAGTGGTTCATCATGGAAATGATTCTCAT
I    CAGAGCAGAAATCATGAGATCTCTCTCTTTTTCAACAAACTTGCAAAAAATATTAAGGAGGAAGGATTGAAAACTGGTTCAACATGGCAATGATCCTGAT
J    CAGAGCAGAAATCATGAGATCCTTCTCTTTTTCAACAAACTTGAAAAAAGGATTAAGGAGGAAGGATTGAAAACTGGTTCATCATGGAAATGATTCTCAT
C    CAGAGCAGAAATCATGAGATCCCTCTCGTTTTCAACAAACTTGCAAAAAAGATTAAGGAGGAAGGATTGAAAACTGGTTCAACATGGCAATGATCCTGAT

                                                                                                    700
A    TGATTCGTATGCCAGCTCACCTTTTTATGATCTGCCATTTCAAAGACTCATGTTTCTGCTATGACCATGACACGATTTAAATCTTTTCAAATGTTTTTAG
H    TGACTAATACATCATCTCACACTTTCATGAGTTCTTCCATTTCAAAGACTCACTTCTCCTATAACCACCACAAGTTGAATCAAAATTTTCAAATGTTTTC
I    TGACTAATACATTATCTCACACTTTCATGAGTTCCTCCATTTCAAAGACTCACTTCTATAACCACCACGAGTTGAATCAAAATTTTCAAATGTTTTCAGC
J    TGACTAATGCATCATCTCACACTTTCATGAGTTCTTCCATTTCAAAGACTCACTTCTATAACCACCACAAGTTGAATCAAAATTTCCAAATGTTTTCAGG
C    TGACTAATACATTATCTCACACTTTCATGAGTTCTTCCATTTCAAAGACTCACTTCTATAACCACGACGTGTTGAATCAAAATTTTCAAATGTTTTCAGC

                                                                                                    800
A    GAGTATTAATCAACATTGTATTCAGCTCTTAAGGCACTAGTCCCTTACAGAGGACCATGCTGACTGATCCATTATCTATTTAAATATTTTTAAAATATTA
H    AGGAGTGTAAAGAAGCATCATGTATACCTGTGCAGGCACTAGTCCTTTACAGATGACCATGCTGATGTCTCCTTTCATCTATTTATTTAAATATTTATTT
I    AGTGTAAAGAAGCGTCGTGTATACCTGTGCAGGCACTAGTACTTTACAGATGACCATGCTGATGTCTCTGTTCATCTATTTATTTAAATATTTATTTAAT
J    AGTGTTAAGAAGCATCGTGTTTACCTGTGCAGGCACTAGTCCTTTACAGATGACCATTCTGATGTCTCCTTTCATCTATTTATTTAAATATTTATTTATT
C    AGTGTAAAGAAGTGTCGTGTATACCTGTGCAGGCACTAGTCCTTTACAGATGACCATTCTGATGTCTCTGTTCATCTTTTGTTTAAATATTTATTTAATT
```

FIG.8C

```
                                                                                                    900
A    TTTATTTAACTATTTATAAAACAACTTATTTTTGTTCATATTATGTCATGTGCACCTTTGCACAGTGGTTAATGTAATAAAATGTGTTCTTTGTATTTGG
H    ATTTAACTATTTTTATTATTTAAATTATTTTTTATGTTAATATCATGTGTACCTTTACATTGTGGTTAATATAACAAATATGTTCTTCATATTTAGCCAA
I    TATTTTTAAGATTTAAATTATTTTTTTTATGTAATATCATGTGTACCTTTACATTGTGGTGAATGTAACAATATATGTTCTTCATATTTAGCCAATATATT
J    TAACTATTTTTATTATTTAAATTATTTTTTATGTAATATCATATGTACCTTTACATTGTGGTTAATGTAACAAATATGTTCTTCATATTTAGCCAATATA
C    ATTTTTAAAATTTATGTAATATCATGAGTCGCTTTACATTGTGGTTAATGTAACAATATATGTTCTTCATATTTAGCCAATATATTAATTTCCTTTTTCA

                                                                                                  1,000
A    TAAATTTATTTTGTGTTGTTCATTGAACTTTTGCTATGGAACTTTTGTACTTGTTTATTCTTTAAAATGAAATTCCAAGCCTAATTGTGCAACCTGATTA
H    TATATTAATTTCCTTTTTCATTAAATTTTTACTATACAAAATTTCTGTGTTTGGTATTT
I    AATTTCCTTTTTCATTAAATTTTTACTATACAAAATTTCTTGAGTTTGTTTATTCTTAAGAATAAAATGTCGAGGCTGACTTTACAACCTGACTTAAAAA
J    TTAATTTCCTTTTT CATTAAATTTTTACTATACAAAATTTCTTGTGTTTGTTTATTTTTTAAGATTAAATGCCAAGCCTGACTGTATAACCTGACTTAA
C    TTAAATTTTTACTATACAAAATTTCTTGTGTTTGTTTATTCTTTAAGATAAAATGCCAAGGCTGACTTTACAACCTGACTTAAAAATAGATGATTTAATT
```

FIG.8D

```
     S1                  S10                 S20      1                  10
A    M A L T F A L L V A L L V L S C K S S C S V G C D L P Q T H S L G S R R T L
H    M A L P F S L M M A L V V L S C K S S C S L G C N L S Q T H S L N N R R T L
I    M A L S F S L L M A V L V L S Y K S I C S L G C D L P Q T H S L G N R R A L
J    M A R S F S L L M V V L V L S K Y S I C S L G C D L P Q T H S L R N R R A L
C    M A L S F S L L M A V L V L S Y K S I * S L G C D L P Q T H S L G N R R A L

               20                  30                  40                  50
A    M L L A Q M R R I S L F S C L K D R H D F G F P Q E E F - G N Q F Q K A E T
H    M L M A Q M R R I S P F S C L K D R H D F E F P Q E E F D G N Q F Q K A Q A
I    I L L A Q M G R I S P F S C L K D R P D F G L P Q E E F D G N Q F Q K T Q A
J    I L L A Q M G R I S P F S C L K D R H E F R F P E E E F D G H Q F Q K T Q A
C    I L L G Q M G R I S P F S C L K D R H D F R I P Q E E F D G N Q F Q K A Q A

                    60                  70                  80                  90
A    I P V L H E M I Q Q I F N L F S T K D S S A A W D E T L L D K F Y T E L Y Q
H    I S V L H E M M Q Q T F N L F S T K N S S A A W D E T L L E K F Y I E L F Q
I    I S V L H E M I Q Q T F N L F S T E D S S A A W E Q S L L E K F S T E L Y Q
J    I S V L H E M I Q Q T F N L F S T E D S S A A W E Q S L L E K F S T E L Y Q
C    I S V L H E M I Q Q T F N L F S T E D S S A A W E Q S L L E K F S T E I Y Q
```

FIG.9A

```
                    100                 110                 120
A   Q L N D L E A C V I Q G V G V T E T P L M K E D S I L A V R K Y F Q R I T L
H   Q M N D L E A C V I Q E V G V E E T P L M N E D S I L A V R K Y F Q R I T L
I   Q L N N L E A C V I Q E V G M E E T P L M N E D S I L A V R K Y F Q R I T L
J   Q L N D L E A C V I Q E V G V E E T P L M N E D F I L A V R K Y F Q R I T L
C   Q L N D L E A C V I Q E V G V E E T P L M N E D S I L A V R K Y F Q R I T L

  130                 140                 150                 160
A   Y L K E K K Y S P C A W E V V R A E I M R S F S L S T N L Q E S L R S K E
H   Y L M E K K Y S P C A W E V V R A E I M R S L S F S T N L Q K R L R R K D
I   Y L T E K K Y S P C A W E V V R A E I M R S L S F S T N L Q K I L R R K D
J   Y L M E K K Y S P C A W E V V R A E I M R S F S F S T N L K K G L R R K D
C   Y L I E R K Y S P C A W E V V R A E I M R S L S F S T N L Q K R L R R K D
```

FIG.9B

FIG.10

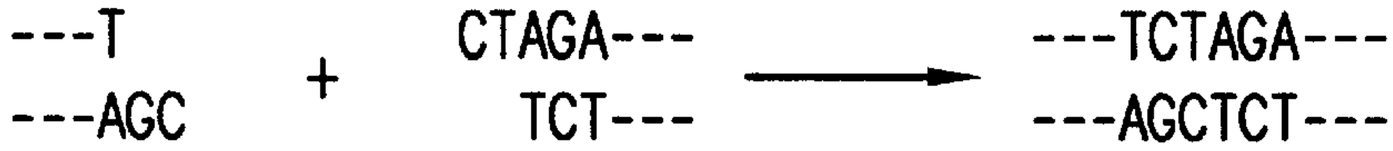


FIG.11

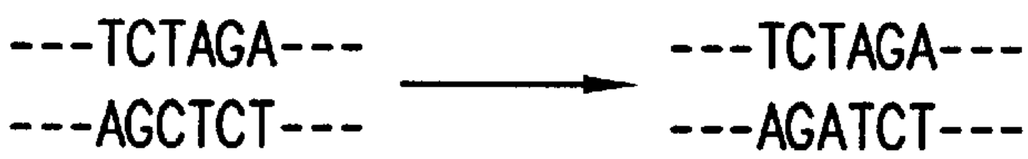


FIG.12

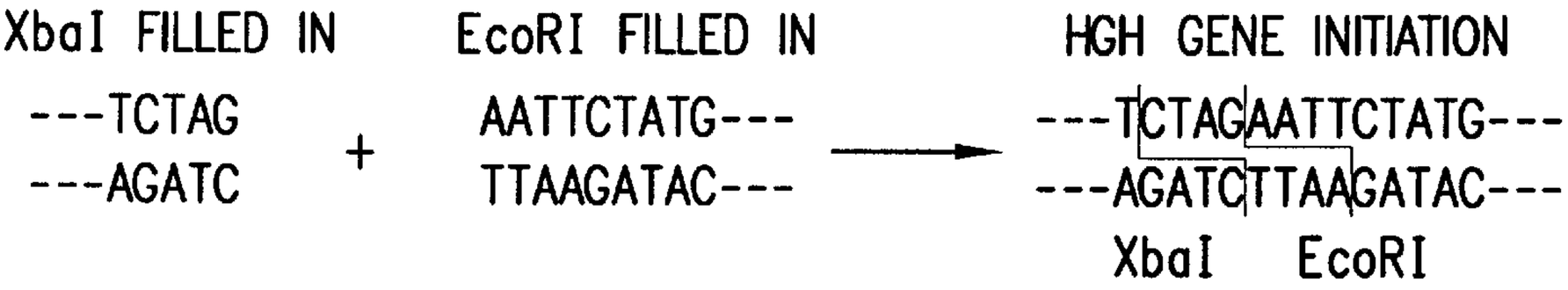


FIG.13

MICROBIAL PRODUCTION OF MATURE HUMAN LEUKOCYTE INTERFERONS

CROSS-REFERENCE TO RELATED APPLICATION

This application is a continuation of application Ser. No. 06/703,148, filed Feb. 19, 1985, now abandoned, which is a division of application Ser. No. 06/256,204 filed Apr. 21, 1981, which is a continuation-in-part of application Ser. No. 06/205,578, filed Nov. 10, 1980, now abandoned, which is a continuation-in-part of application Ser. No. 06/184,909, filed Sep. 8, 1980, now abandoned, which is a continuation-in-part of application Ser. No. 06/164,986, filed Jul. 1, 1980, now abandoned.

FIELD OF THE INVENTION

This invention relates to the microbial production, via recombinant DNA technology, of human leukocyte interferons for use in the treatment of viral and neoplastic diseases, and to the means and end products of such production.

BACKGROUND OF THE INVENTION

The publications and other materials referred to herein to illuminate the background of the invention and, in particular cases, to provide additional detail respecting its practice are incorporated herein by reference and, for convenience, are numerically referenced in the following text and respectively grouped in the appended bibliography.

Leukocyte Interferon

Human leukocyte interferon was first discovered and prepared in the form of very crude precipitates by Isaacs and Lindenmann (3). Efforts to purify and characterize the material have been ongoing since that time, and have led to the preparation of relatively homogeneous leukocyte interferons derived from normal or leukemic (chronic myelogenous leukemia or "CML") donors' leukocytes (4). These interferons are a family of proteins characterized by a potent ability to confer a virus-resistant state in their target cells (1,2). In addition, interferon can act to inhibit cell proliferation and modulate immune response. These properties have prompted the clinical use of leukocyte interferon as a therapeutic agent for the treatment of viral infections and malignancies.

Leukocyte interferons have been purified to essential homogeneity (7,8), and reported molecular weights range from about 17,500 to about 21,000. The specific activity of these preparations is remarkably high, 2×10^8 to 1×10^9 units/mg protein, but yields from cell culture methods have been discouragingly low. Nevertheless, advances in protein sequencing techniques have, in our hands, permitted the determination of partial amino acid sequences (4). Elucidation of the glycosylation of various leukocyte interferons is not at present complete, but it is now clear (by virtue of the work reported infra) that differences in glycosylation among family members does not alone account for the spectrum of molecular weights observed. Instead, the leukocyte interferons differ markedly in amino acid composition and sequence, and amino acid homology is, in some cases, less than 80 percent.

While isolation from donor leukocytes has provided sufficient material for partial characterization and limited clinical studies with homogeneous leukocyte interferon, it is a totally inadequate source for the amounts of interferon needed for large scale clinical trials and for broad scale prophylactic and/or therapeutic use thereafter. Indeed, presently clinical investigations employing human leukocyte-derived interferons in antitumor and antiviral testing have principally been confined to crude (<1 percent pure) preparations of the material, and long lead times for the manufacture of sufficient quantities, even at unrealistic price levels, have critically delayed investigation on an expanded front.

Recombinant DNA Technology

With the advent of recombinant DNA technology, the controlled microbial production of an enormous variety of useful polypeptides has become possible. Already in hand are bacteria modified by this technology to permit the production of such polypeptide products such as somatostatin (5), the (component) A and B chains of human insulin (9) and human growth hormone (18). More recently, recombinant DNA techniques have been used to occasion the bacterial production of proinsulin and thymosin alpha 1, an immune potentiating substance produced by the thymus.

Other workers have reported on the obtention of DNA coding for human leukocyte interferon and to resultant proteins having leukocyte interferon activity—cf. Nagata et al., *Nature* 284, 316 (1980); Mantei et al., *Gene* 10, 1 (1980). See also Taniguchi et al., *Nature* 285, 547 (1980).

The workhorse of recombinant DNA technology is the plasmid, a non-chromosomal loop of double-stranded DNA found in bacteria and other microbes, oftentimes in multiple copies per cell. Included in the information encoded in the plasmid DNA is that required to reproduce the plasmid in daughter cells (i.e., a "replicon") and ordinarily, one or more selection characteristics such as, in the case of bacteria, resistance to antibiotics which permit clones of the host cell containing the plasmid of interest to be recognized and preferentially grown in selective media. The utility of plasmids lies in the fact that they can be specifically cleaved by one or another restriction endonuclease or "restriction enzyme", each of which recognizes a different site on the plasmidic DNA. Thereafter heterologous genes or gene fragments may be inserted into the plasmid by endwise joining at the cleavage site or at reconstructed ends adjacent to the cleavage site. DNA recombination is performed outside the cell, but the resulting "recombinant" plasmid can be introduced into it by a process known as transformation and large quantities of the heterologous gene-containing recombinant plasmid obtained by growing the transformant. Moreover, where the gene is properly inserted with reference to portions of the plasmid which govern the transcription and translation of the encoded DNA message, the resulting expression vehicle can be used to actually produce the polypeptide sequence for which the inserted gene codes, a process referred to as expression. Expression is initiated in a region known as the promoter which is recognized by and bound by RNA polymerase. In some cases, as in the tryptophan or "trp" promoter preferred in the practice of the present invention, promoter regions are overlapped by "operator" regions to form a combined promoter-operator. Operators are DNA sequences which are recognized by so-called repressor proteins which serve to regulate the frequency of transcription initiation at a particular promoter. The polymerase travels along the DNA, transcribing the information contained in the coding strand from its 5' to 3' end into messenger RNA which is in turn translated into a polypeptide having the amino acid sequence for which the DNA codes. Each amino acid is encoded by a nucleotide triplet or "codon" within what may for present purposes be referred to as the "structural gene", i.e. that part which encodes the amino acid sequence of the expressed product. After binding to the promoter, the RNA polymerase first transcribes nucleotides encoding a ribosome binding site,

then a translation initiation or "start" signal (ordinarily ATG, which in the resulting messenger RNA becomes AUG), then the nucleotide codons within the structural gene itself. So-called stop codons are transcribed at the end of the structural gene whereafter the polymerase may form an additional sequence of messenger RNA which, because of the presence of the stop signal, will remain untranslated by the ribosomes. Ribosomes bind to the binding site provided on the messenger RNA, in bacteria ordinarily as the mRNA is being formed, and themselves produce the encoded polypeptide, beginning at the translation start signal and ending at the previously mentioned stop signal. The desired product is produced if the sequences encoding the ribosome binding site are positioned properly with respect to the AUG initiator codon and if all remaining codons follow the initiator codon in phase. The resulting product may be obtained by lysing the host cell and recovering the product by appropriate purification from other bacterial protein.

We perceived that application of recombinant DNA technology would be the most effective way of providing large quantities of leukocyte interferon which, despite the absence in material so produced of the glycosylation characteristic of human-derived material, could be employed clinically in the treatment of a wide range of viral and neoplastic diseases.

More particularly, we proposed and have since succeeded in producing mature human leukocyte interferon microbially, by constructing one or more genes therefor which could then be inserted in microbial expression vehicles and expressed under the control of microbial gene regulatory controls.

Our approach to obtaining a first leukocyte gene involved the following tasks:

1. Partial amino acid sequences would be obtained by characterization of leukocyte interferon purified to essential homogeneity, and construct sets of synthetic DNA probes constructed whose codons would, in the aggregate, represent all the possible combinations capable of encoding the partial amino acid sequences.

2. Bacterial colony banks would be prepared containing cDNA from induced messenger RNA. Other induced mRNA that had been radio-labelled would be hybridized to plasmid cDNA from this bank. Hybridizing mRNA would be eluted and tested for translation into interferon in oocyte assay. Plasmid DNA from colonies shown positive for interferon in this manner would be further tested for hybridization to probes made as described in (1) above.

3. Parallel to the approach in part (2) above, induced mRNA-derived cDNA in plasmids would be used to form an independent bank of transformant colonies. The probes of part (1) would be used to prime the synthesis of radio-labelled single stranded cDNA for use as hybridization probes. The synthetic probes would hybridize with induced mRNA as template and be extended by reverse transcription to form induced, radio-labelled cDNA. Clones from the colony bank that hybridized to radio-labelled cDNA obtained in this manner would be investigated further to confirm the presence of a full-length interferon encoding gene. Any partial length putative gene fragment obtained in parts (1) or (2) would itself be used as a probe for the full-length gene.

4. The full-length gene obtained above would be tailored, using synthetic DNA, to eliminate any leader sequence that might prevent microbial expression of the mature polypeptide and to permit appropriate positioning in an expression vehicle relative to start signals and the ribosome binding site of a microbial promoter. Expressed interferon would be purified to a point permitting confirmation of its character and determination of its activity notwithstanding the absence of glycosylation.

5. The interferon gene fragment prepared in the foregoing fashion could itself be used in probing, by hybridization, for other partially homologous leukocyte interferon species.

BRIEF SUMMARY OF INVENTION

We have discovered and, through recombinant DNA technology, enabled the microbial production in high yield of the family of homologous leukocyte interferons (sans glycosylation) as mature polypeptides, essentially unaccompanied by the corresponding presequence or any portion thereof. These may be directly expressed, recovered and purified to levels fitting them for use in the treatment of viral and malignant diseases of animals and man. Family members so far expressed have proven efficacious in in vitro testing and, in the first such demonstration of its kind, in in vivo testing as well, the latter involving the first mature leukocyte interferon to have been microbially produced. The invention comprises the interferons so produced and means of producing them.

Reference herein to the expression of a "mature leukocyte interferon," connotes the bacterial or other microbial production of an interferon molecule unaccompanied by associated glycosylation and the presequence that (as we have discovered) immediately attends mRNA translation of a human leukocyte interferon genome. Mature leukocyte interferon, according to the present invention, is immediately expressed from a translation start signal (ATG) just before the first amino acid codon of the natural product, in which event the mature polypeptide includes the methionine for which ATG codes without essentially altering its character, or the microbial host may process the translation product to delete the initial methionine. Mature leukocyte interferon could be expressed together with a conjugated protein other than the conventional leader, the conjugate being specifically cleavable in an intra- or extracellular environment. See British Patent Publication No. 2007676A. Finally, the mature interferon could be produced in conjunction with a microbial "signal" peptide which transports the conjugate to the cell wall, where the signal is processed away and the mature polypeptide secreted.

Particular leukocyte interferon proteins hereof have been defined by means of determined DNA gene and deductive amino acid sequencing—cf. FIGS. **3**, **4**, **8** and **9**, for example. It will be understood that for these particular interferons, indeed all of the family of leukocyte interferon proteins embraced herein, natural allelic variations exist and occur from individual to individual. These variations may be demonstrated by (an) amino acid difference(s) in the overall sequence or by deletions, substitutions, insertions, inversions or additions of (an) amino acid(s) in said sequence. For each leukocyte interferon protein hereof, labelled LeIF A, LeIF B . . . LeIF J, etc., such allelic variations are included within the scope of the label or term defining such, and thus, this invention.

BRIEF DESCRIPTION OF THE DRAWINGS

FIG. **1** depicts two series of synthetic deoxynucleotides designated T-1 and T-13 designed to prime cDNA synthesis from leukocyte interferon ("Le-IF") mRNA. Amino acid sequences are given for peptide 1 and a portion of peptide 13 derived from a tryptic digest of human Le-IF β (4). All potential mRNA sequences coding for these peptides are-shown, as are the corresponding DNA sequences. Here and throughout, the letters A, T, C, G and U respectively connote

the nucleotides containing the bases adenine, thymine, guanine, cytosine and uracil, and polynucleotides are depicted as reading from the 5' (left) in the 3' (right) direction and, where double stranded ("d.s.") DNA is depicted, vice-versa for the bottom or non-coding strand.

FIG. **2** is an autoradiogram showing hybridization of potential Le-IF plasmids with ^{32}P-labelled synthetic deoxyoligonucleotides.

FIGS. **3**A–**3**E depict the nucleotide sequence (coding strand) of eight gene fragments isolated as candidates for use in the expression of leukocyte interferons, respectively designated "A" through "H". The ATG translational initiation codon and the termination triplet for each LeIF is underlined. The stop codons or termination triplets are followed by 3' untranslated regions. The included full length gene for Le-IF "A" is missing one codon found in the others depicted, as indicated in the third "A" line of FIG. **3**B. 5' untranslated regions precede the leader sequences. As isolated, fragment "E" lacked the full presequence or leader, but included the entire gene for the putative mature Le-IF "E". Fragment G as isolated lacked the full coding sequence. The nucleotide sequence in the rows labeled "LeIF A" (SEQ ID NO:1) encodes a 188 amino acid translation product (SEQ ID NO:2). The nucleotide sequence in the rows labeled "LeIF B" (SEQ ID NO:3) encodes a 189 amino acid translation product (SEQ ID NO:4). The nucleotide sequence in the rows labeled "LeIF C" (SEQ ID NO:5) encodes a 189 amino acid translation product (SEQ ID NO:6). The nucleotide sequence in the rows labeled "LeIF D" (SEQ ID NO:7) encodes a 189 amino acid translation product (SEQ ID NO:8). The nucleotide sequence in the rows labeled "LeIF E" (SEQ ID NO:9) encodes fragments of a translation product: nucleotides 1–186 encode a 62 amino acid fragment (SEQ ID NO:10), nucleotides 190–234 encode a 15 amino acid fragment (SEQ ID NO:62), nucleotides 250–273 encode an 8 amino acid fragment (SEQ ID NO:63), nucleotides 277–294 encode a 6 amino acid fragment (SEQ ID NO:64), nucleotides 298–420 encode a 41 amino acid fragment (SEQ ID NO:65), and nucleotides 424–534 encode a 37 amino acid fragment (SEQ ID NO:66). The nucleotide sequence in the rows labeled "LeIF F" (SEQ ID NO:11) encodes a 189 amino acid translation product (SEQ ID NO: 12). The nucleotide sequence in the rows labeled "LeIF G" (SEQ ID NO:13) encodes a partial amino acid translation product (SEQ ID NO:14). The nucleotide sequence in the rows labeled "LeIF H" (SEQ ID NO:15) encodes a 189 amino acid translation product (SEQ ID NO:16). Nucleotides 126–623 of SEQ ID NO:15 encode a 166 residue mature amino acid translation product (SEQ ID NO:56). The nucleotide sequence in the rows labeled "LeIF A" (SEQ ID NO:1) encodes a 188 amino acid translation product (SEQ ID NO:2). The nucleotide sequence in the rows labeled "LeIF B" (SEQ ID NO:3) encodes a 189 amino acid translation product (SEQ ID NO:4). The nucleotide sequence in the rows labeled "LeIF C" (SEQ ID NO:5) encodes a 189 amino acid translation product (SEQ ID NO:6). The nucleotide sequence in the rows labeled "LeIF D" (SEQ ID NO:7) encodes a 189 amino acid translation product (SEQ ID NO:8). The nucleotide sequence in the rows labeled "LeIF E" (SEQ ID NO:9) encodes fragments of a translation product: nucleotides 1–186 encode a 62 amino acid fragment (SEQ ID NO:10), nucleotides 190–234 encode a 15 amino acid fragment (SEQ ID NO:62), nucleotides 250–273 encode an 8 amino acid fragment (SEQ ID NO:63), nucleotides 277–294 encode a 6 amino acid fragment (SEQ ID NO:64), nucleotides 298–420 encode a 41 amino acid fragment (SEQ ID NO:65), and nucleotides 424–534 encode a 37 amino acid fragment (SEQ ID NO:66). The nucleotide sequence in the rows labeled "LeIF F" (SEQ ID NO:11) encodes a 189 amino acid translation product (SEQ ID NO:12). The nucleotide sequence in the rows labeled "LeIF G" (SEQ ID NO:13) encodes a partial amino acid translation product (SEQ ID NO:14). The nucleotide sequence in the rows labeled "LeIF H" (SEQ ID NO:15) encodes a 189 amino acid translation product (SEQ ID NO:16). Nucleotides 126–623 of SEQ ID NO:15 encode a 166 residue mature amino acid translation product (SEQ ID NO:56).

FIGS. **4**A–**4**B are a comparison of eight LeIF protein sequences predicted from nucleotide sequences. The one letter abbreviations recommended by the IUPAC-IUB Commission on Biochemical Nomenclature are used: A, alanine; C, cysteine; D, aspartic acid; E, glutamic acid; F, phenylalanine; G, glycine; H, histidine; I, isoleucine; K, lysine; L, leucine; M, methionine; N, asparagine; P, proline; Q, glutamine; R, arginine; S, serine; T, threonine; V, valine; W, tryptophan; and Y, tyrosine. The numbers refer to amino acid position (S refers to signal peptide). The dash in the 165 amino acid LeIF A sequence at position 44 is introduced to align the LeIF A sequence with the 166 amino acid sequences of the other LeIFs. The LeIF E sequence was determined by ignoring the extra nucleotide (position 187 of FIG. **3**B) in its coding region. The asterisks indicate in-phase termination codons. The amino acid sequence in the rows labeled "LeIF A" is a 188 residue LeIF A polypeptide (SEQ ID NO:17) with an intact signal peptide. The amino acid sequence labeled S1–S23 is the signal peptide, and the amino acid sequence labeled 1–166 is the mature LeIF A polypeptide (SEQ ID NO:45).

The amino acid sequence in the rows labeled "LeIF B" is a 189 residue LeIF B polypeptide (SEQ ID NO:18) with a portion of a signal peptide. The amino acid sequence labeled S1–S23 is the signal peptide, and the amino acid sequence labeled 1–166 is the mature LeIF B polypeptide (SEQ ID NO:46).

The amino acid sequence in the rows labeled "LeIF C" is a 189 residue LeIF C polypeptide (SEQ ID NO:19) with an intact signal peptide. The amino acid sequence labeled S1–S23 is the signal peptide, and the amino acid sequence labeled 1–166 is the mature LeIF C polypeptide (SEQ ID NO:47). The amino acid sequence in the rows labeled "LeIF D" is a 189 residue LeIF D polypeptide (SEQ ID NO:20) with an intact signal peptide. The amino acid sequence labeled S1–S23 is the signal peptide, and the amino acid sequence labeled 1–166 is the mature LeIF D polypeptide (SEQ ID NO:48). The amino acid sequences in the rows labeled "LeIF E" are fragments of a LeIF E polypeptide including the amino acid sequence labeled S20–101 (SEQ ID NO:21), the amino acid sequence labeled 103–154 (SEQ ID NO:67), and the amino acid sequence labeled 156–166 (SEQ ID NO:68). The amino acid sequence in the rows labeled "LeIF F" is a 189 residue LelF F polypeptide (SEQ ID NO:22) with an intact signal peptide. The amino acid sequence labeled S1–S23 is the signal peptide, and the amino acid sequence labeled 1–166 is the mature LeIF F polypeptide (SEQ ID NO:49). The amino acid sequence in the rows labeled "LeIF G" is a 133 residue fragment of a LeIF G polypeptide (SEQ ID NO:23).

The amino acid sequence in the rows labeled "LeIF H" is a 189 residue LeIF H polypeptide (SEQ ID NO:24) with an intact signal peptide. The amino acid sequence labeled S1–S23 is the signal peptide, and the amino acid sequence labeled 1–166 is the mature LeIF H polypeptide (SEQ ID NO:50). Amino acids common to all LeIFs (excluding the

pseudogene LeIF E) are also shown in the rows labeled "All". The underlined residues are amino acids which are also present in human fibroblast interferon.

FIG. **5** depicts restriction endonuclease maps of the eight types of LeIF cloned cDNAs (A through H). The hybrid plasmids were constructed by the dC:dG tailing method Goeddel, D. V. et al, *Nature* 287, 411–416 (1980). Therefore, the cDNA inserts can be exised using Pst I. The lines at the end of each cDNA insert represent the flanking homopolymeric dC:dG tails. The positions of Pvu II, Eco RI and Bgl II restriction sites are indicated. Shaded regions of the figure represent the coding sequences of mature LeIFs; the cross-hatched regions indicate signal peptide coding sequences; and the open regions show 3' and 5' noncoding sequences.

FIG. **6** schematically depicts the construction of a gene coding for the direct microbial synthesis of mature Le-IF A. Restriction sites and residues are as shown ("Pst I", etc.). The term "b.p." connotes "base pair."

FIGS. **7**(*a* and *b*) (not to scale) schematically depicts a restriction map of two gene fragments employed in expressing the mature leukocyte interferon Le-IF B. The codon sequences indicated are the coding strand terminii resulting from digestion with the restriction enzyme Sau 3a in the two cases shown.

FIGS. **8**A–**8**D provide the DNA sequences of the five LeIF proteins hereof, including types I and J. The nucleotide sequence (SEQ ID NO:25) in the rows labeled "A" encodes a 188 amino acid translation product (SEQ ID NO:26). The nucleotide sequence (SEQ ID NO:27) in the rows labeled "H" encodes a 189 amino acid translation product (SEQ ID NO:28). Nucleotides 180–677 of SEQ ID NO:27 encode a mature 166 amino acid translation product (SEQ ID NO:58). The nucleotide sequence (SEQ ID NO:29) in the rows labeled "I" encodes a 189 amino acid translation product (SEQ ID NO:30). Nucleotides 182–679 of SEQ ID NO:29 encode a mature 166 amino acid translation product (SEQ ID NO:59). The nucleotide sequence (SEQ ID NO:31) in the rows labeled "J" encodes a 189 amino acid translation product (SEQ ID NO:32). The nucleotide sequence (SEQ ID NO:33) in the rows labeled "C" encodes two amino acid translation products. Nucleotides 110–166 of SEQ ID NO:33 encode a 19 amino acid translation product (SEQ ID NO:69), and nucleotides 170–676 of SEQ ID NO:33 encode a 169 amino acid translation product (SEQ ID NO:34). Nucleotides 179–676 of SEQ ID NO:33 encode a mature 166 amino acid translation product (SEQ ID NO:57).

FIGS. **9**A–**9**B provide the amino acid (see FIGS. **4**A–**4**B above for the corresponding one letter abbreviations) sequences of five LeIF proteins hereof. The asterisk indicates a termination codon and the hyphen, a deletion or gap in the sequence. The amino acid sequence in the rows labeled "A" is a 188 residue polypeptide (SEQ ID NO:35) with an intact signal peptide. The amino acid sequence labeled S1–S23 is the signal peptide, and the amino acid sequence labeled 1–166 is the mature polypeptide (SEQ ID NO:53).

FIG. **10** schematically depicts filling in of 2 of the 4 nucleotides complementary to the 5' protruding end of the XbaI cleavage site.

FIG. **11** schematically depicts ligation of the Taq I protruding end of the Eco RI-Taq I fragment to the XbaI remaining protruding end of the fragment from pHS32.

FIG. **12** schematically depicts regeneration of the XbaI site via *E. coli* catalyzed DNA repair and replication.

FIG. **13** schematically depicts blunt end ligation of an XbaI filed in fragment with an Eco RI filled in fragment to recreate both the XbaI and the Eco RI site.

DESCRIPTION OF THE PREFERRED EMBODIMENTS

A. Microorganisms Employed

The work described involved use of two microorganisms: *E. coli* x1776, as described in (11), and *E. coli* K-12 strain 294 (end A, thi^-, hsr^-, hsm_k^+), as described in (12). Each has been deposited with the American Type Culture Collection, which isolated at 12301 parklawn Drive, Rockville, Mo. 20852 respectively ATCC accession nos. 31537 and 31446. All recombinant DNA work was performed in compliance with applicable guidelines of the National Institutes of Health.

The invention, in its most preferred embodiments, is described with reference to *E. coli*, including not only strains *E. coli* x1776 and *E. coli* K-12 strain 294, defined above, but also other known *E. coli* strains such as *E. coli* B, or other microbial strains many of which are deposited and (potentially) available from recognized microorganism depository institutions, such as the American Type Culture Collection (ATCC)—cf. the ATCC catalogue listing. See also German Offenlegungsschrift 2644432. These other microorganisms include, for example, Bacilli such as *Bacillus subtilis* and other enterobacteriaceae among which can be mentioned as examples *Salmonella typhimurium* and *Serratia marcesans,* utilizing plasmids that can replicate and express heterologous gene sequences therein. Yeast, such as *Saccharomyces cerevisiae,* may also be employed to advantage as host organism in the preparation of the interferon proteins hereof by expression of genes coding therefor under the control of a yeast promoter. (See the copending U.S. patent application Ser. No. 06,237,913 of Hitzeman et al., filed Feb. 25, 1981, assignee Genentech, Inc. et al., which is incorporated herein by reference.)

B. Source of Le-IF mRNA

Le-IF mRNA may be obtained from human leukocytes, ordinarily those of patients with chronic nyelogenous leukemia, that have been induced to produce interferon with Sendai or NDV virus, as described in (4). A particularly preferred source, and that used in the work reported herein, is a cell line designated KG-1 derived from a patient with acute myelogenous leukemia. The cell line, described by Koeffler, H. P. and Golde, D. W., *Science* 200, 1153 (1978), reference (15), grows readily in a culture medium comprising RPMI 1640 plus 10 FCS (heat-inactivated), 25 mM Hepes buffer and 50 μg/ml of gentamicin, and is subcultured 1 to 3 split two times a week. Cells may be frozen from the foregoing growth medium plus 10 dimethyl sulfoxide. KG-1 has been deposited with the American Type Culture Collection, ATCC accession no. CRL 8031.

C. Messenger RNA Purification from KG-1 Cells

KG-1 cells were induced to produce interferon (and leukocyte interferon mRNA) with Sendai or NDV following the procedure described by Rubinstein et al., *Proc. Natl. Acad. Sci. USA* 76, 640 (1979) and Familetti et al., *Methods in Enzymology,* (1981) (in press) and (4). Cells were harvested 5 hours after induction and RNA prepared by the guanidine thiocyanate-guanidine hydrochloride procedure (14). RNA from uninduced cells was isolated in the same manner. Oligo (dT)—cellulose chromatography and sucrose gradient ultracentrifugation was used to obtain the 12 S sucrose gradient fraction of poly (A) RNA as described (16,21). This mRNA had an interferon titer of 8000–10,000 units per microgram in the *Xenopus laevis* oocyte assay (6).

D. Preparation of Colony Banks Containing Le-IF cDNA Sequences

5 μg of mRNA was used to prepare double stranded cDNA by standard, procedures (17,18). The cDNA was size frac-

tionated by electrophoresis on a 6 polyacrylamide gel and 230 ng of material ranging in size from 500 to 1500 base pairs were recovered by electroelution. A 100 ng portion of this cDNA was tailed with deoxyC residues (19), annealed with 470 ng of plasmid pBR322 (20) which had been tailed with deoxy G residues at the Pst I site, and used to transform *E. coli* X1776. Approximately 130 tetracycline resistant, ampicillin sensitive transformants were obtained per ng of cDNA.

In a second similar experiment, approximately 1000 tetracycline resistant, ampicillin sensitive *E. coli* 294 transformants were obtained per ng of cDNA. In this case size fractionated cDNA material ranging in size from 600 to 1300 b.p. was recovered by electroelution for deoxyC tailing.

E. Preparation of Synthetic Oligonucleotides

The amino acid sequences of several tryptic fragments of human leukocyte interferon were determined (4). This information permitted the design of synthetic deoxyoligonucleotides potentially complementary to different regions of LeIF mRNA. The two tryptic peptides T1 and T13 were selected because they had amino acid sequences requiring the synthesis of only 12 and 4 undecamers, respectively, to acount for all possible coding sequences (FIG. **1**.). Four sets of deoxyoligonucleotide probes were synthesized for each sequence, containing either three (T-1A, B, C, D) or one (T-13A, B, C, D) oligonucleotide each. The indicated complementary deoxyoligonucleotides 11 bases long were chemically synthesized by the phosphotriester method (24). Four individual probes were prepared in the T-13 series. The twelve T-1 probes were prepared in four pools of three probes as shown in FIG. **1**.

F. Isolation of Partial Le-IF Gene Fragment Containing Plasmid No. 104

Transformants of *E. coli* x1776 were screened by the colony hybridization procedure (27) using ^{32}P-labelled (32) induced mRNA as probe. Unlabelled mRNA from uninduced cells was mixed with the probe at a ratio of 200 to 1 to compete with uninduced mRNA present in the ^{32}P-labelled preparation. Hybridization of labelled mRNA should occur preferentially to colonies containing induced sequences. Three classes of transformants were obtained. (1) 2–3 of the colonies hybridized to ^{32}P-mRNA very strongly, (2) 10 hybridized significantly less than class 1, and (3) the remainder gave no detectable hybridization signal. This 3rd class was eliminated from further screening.

The positive colonies were examined for the presence of interferon-specific sequences by an assay which depends upon hybridization of interferon mRNA specifically to plasmid DNA. Initially, 60 strong positive colonies (class 1) were grown individually in 100 ml of M-9 broth (42) supplemented with tetracycline (20 μg/ml), diaminopimelic acid (100 μg/ml), thymidine (20 μg/ml), and d-biotin (1 μg/ml). Ten cultures were pooled and plasmid DNA was isolated from the six pools as described earlier (34,35). Ten μg of each plasmid DNA pool were cleaved with HindIII, denatured and covalently bound to DBM paper (36). One μg of purified mRNA from induced cells was hybridized to each filter. Unhybridized mRNA was removed by washing. The specifically hybridized mRNA was eluted and translated in *Xenopus laevis* oocytes. By this assay, all six pools were negative. Five pools of ten colonies each and one pool of nine colonies were made from 59 weakly positive colonies, (class 2) and plasmids were prepared from the pools and examined as above. Among the six pools tested, one (K10) hybridized to interferon mRNA at levels significantly above background levels each time it was tested. In order to identify the specific interferon cDNA clone plasmid DNAs were prepared from the 9 colonies of pool K10 and examined individually. Two of the nine plasmids (No. 101 and No. 104) bound interferon mRNA well above background levels.

G. Preparation and Use of cDNA Probes Obtained by Synthetic Oligonucleotide Priming of Induced mRNA; Identification of Colonies pL 1-30

A rapid plasmid isolation procedure (22) was used to prepare 1 μg of plasmid DNA from each of 500 individual *E. coli* 294 transformants. Each DNA sample was denatured and applied to nitrocellulose filters in triplicate following a published procedure (23).

The four individual probes of the T-13 series and the twelve T-1 probes prepared in four pools of three primers each were used to prime the synthesis of radiolabelled single stranded cDNA for use as hybridization probes. The template mRNA was either the 12S RNA from Sendai-induced KG-1 cells (8000 units IF activity per μg) or total poly (A) mRNA from uninduced leukocytes (<10 units per μg). ^{32}P-labelled cDNA was prepared from these primers using published reaction conditions (25). The 60 μl reactions were performed in 20 mM Tris-HC1 (pH8.3), 20 mM KC1, 8 mM $MgCl_2$, 30 mM β-merceptoethanol. Reactions included one μg of each primer (i.e. 12 μg total for T-1 series, 4 μg total for T-13 series), 2 μg of "induced" 12S fraction mRNA (or 10 μg of uninduced poly (A) mRNA), 0.5 mM dATP, dGTP, dTTP, 200 μCi (α^{32}P)dCTP (Amersham, 2–3,000 Ci/mmole), and 60 units reverse transcriptase (Bethesda Research Laboratories). Product was separated from unincorporated label by gel filtration on a 10 ml Sephadex G-50 column, treated with 0.3N NaOH for 30' at 70° C. to destroy RNA, and neutralized with HC1. Hybridizations were performed as described (23).

The three sets of nitrocellulose filters containing the 500 plasmid samples were hybridized with a) induced cDNA primed with the T-1 set of primers, b) T-13 primed induced cDNA, and c) uninduced cDNA prepared by using both sets of primers. Clones were considered positive if they hybridized more strongly to one or both of the induced cDNA probes than to the total uninduced probe. Thirty "positive" clones (pL1–pL30) were selected from the 500 for further analysis.

H. Selection of Additional "Positive" Colonies pL31–39 Using a Restriction Fragment of Plasmid 104

A unique 260 b.p. BglII restriction fragment isolated from the plasmid 104 clone was labelled by a published procedure (26) with ^{32}P and used as probe to independently screen 400 *E. coli* 294 transformants by an in situ colony screening procedure (27). Nine colonies (pL31–pL39) were identified which hybridized to different extents with this probe. In addition, the labelled 260 bp fragment was used to independently screen 4000 *E. Coli* 294 transformants in the same manner. 50 colonies were identified which hybridized to different extents with this probe. One contained the Le-IF G fragment, one contained the Le-IF H fragment, and one contained a fragment designated Le-IF H1, an apparent allele of Le-IF H. The hybrid plasmids which result are designated "pLe-IF H", etc.

I. Isolation and DNA Sequencing of a First Full-Length Le-IF Gene Fragment from pL1–39

Plasmid DNA was prepared from all 39 potential Le-IF cDNA clones and rescreened with the same 260 b.p. DNA probe using the dot hybridization procedure (23). Three plasmids (pL4, pL31, pL34) gave very strong hybridization signals, four (pL13, pL30, pL32, pL36) hybridized moderately, and three (pL6, pL8, pL14) hybridized weakly with the probe.

The 39 potential Le-IF cDNA recombinant plasmids were also screened by using ^{32}P-labelled synthetic undecamers (individual T-1 primer pools or individual T-13 primers) directly as hybridization probes. The hybridization conditions were chosen such that perfect base pairing should be required for detectable hybridization signals (28). Thus, plasmid DNA from the 39 clones was prepared by a standard cleared lysate procedure (29) and purified by Biorad Agarose A-50 column chromatography. Samples (3 μg) of each prep were linearized by treatment with Eco RI, denatured in alkali and spotted on 2 separate nitrocellulose filters (1.5 μg per spot) (23). Individual synthetic deoxyoligonucleotide primers and primer pools were phosphorylated with (γ^{32}P) ATP as follows: 50 pmoles of oligonucleotide and 100 pmoles of (γ^{32}P)ATP (New England Nuclear, 2500 Ci/mmole) were combined in 30 μl of 60 mM Tris-HCl (pH8), 10 mM $MgCl_2$, 15 mM β-merceptothanol. 2 units of T4 polynucleotide kinase were added and, after 30' at 37° C., ^{32}P labelled primers were purified by chromatography on 10 ml Sephadex G-50 columns. Hybridizations were performed using 10^6 cpm of primer T-13C or 3×10^6 cpm of primer pool T-1C. The hybridizations were performed at 15° C. for 14 hours in 6×SSC, 10× Denhardt's solution, as described by Wallace et al. (28). Filters were washed for 5' (3 times) at 0° C. in 6×SSC, dried, and exposed to x-ray film. Results are shown in FIG. **2** for ^{32}P primer pool T-13C and primer T-1C.

Plasmid DNA from clone 104 was found to give significant hybridization with primer pool T-1C and primer T-13C, but no detectable hybridization with the other undecamers. As shown in FIG. **2**, several of the 39 potential Le-IF plasmids (pL2, 4, 13, 17, 20, 30, 31, 34) also hybridized with both of these probes. Restriction analysis showed that only one of these plasmids, pL31, also contained a 260 b.p. internal Bgl II fragment. Pst I digestion of pL31 showed the size of the cDNA insert to be approximately 1000 base pairs.

The entire Pst I insert of pL31 was sequenced by both the Maxam-Gilbert chemical method (30) and by dideoxy chain termination procedure (31) after subcloning Sau 3a fragments into an M13 vector. The DNA sequence is shown ("A") in FIGS. **3**A–**3**E. The appropriate translational reading frame could be predicted from protein sequence information in hand (4), the known range of Le-IF molecular weights and the relative incidence of stop triplets in the three possible reading frames, and that in turn permitted prediction of the entire Le-IF amino acid sequence, including a pre- or signal peptide. The first ATG translational initiation codon is found 60 nucleotides from the 5' end of the sequence and is followed, 188 codons later, by a TGA termination triplet; there are 342 untranslated nucleotides at the 3' end followed by a poly (A) sequence. The putative signal peptide (presumably involved in the secretion of the mature LeIF from leukocytes) is 23 amino acids long. The 165 amino acids constituting the mature LeIF have a calculated MW of 19,390. We have termed the Le-IF encoded by pL31 "Le-IF A." It can be seen from the sequence data ("A") in FIG. **5** that tryptic peptides T1 and T13 of Le-IF B (4) (FIG. **1**) correspond to amino acids 145–149 and 57–61 respectively of the Le-IF A. The actual DNA coding sequences found in these two regions are those represented by primer pool T1-C and primer T13-C as the data shown in FIG. **2** suggested.

J. Direct Expression of a First Mature Leukocyte Interferon

1. Generally

The procedure followed to express Le-IF A directly as a mature interferon polypeptide was a variant of that earlier employed for human growth hormone (18), insofar as it involved the combination of synthetic (N-terminal) and complementary DNAs.

As shown in FIG. **6**, a Sau 3a restriction endonuclease site is conveniently located between codons 1 and 2 of Le-IF A. Two synthetic deoxyoligonucleotides were designed which incorporate an ATG translational initiation codon, restore the codon for amino acid 1 (cysteine), and create an Eco RI sticky end. These oligomers were ligated to a 34 b.p. Sau 3a-Ava II fragment of pL31. The resulting 45 b.p. product was ligated to two additional DNA fragments to construct an 865 base pair synthetic-natural hybrid gene which codes for Le-IF A and which is bounded by Eco RI and Pst I restriction sites. This gene was inserted into pBR322 between the Eco RI and Pst I sites to give the plasmid pLe-IF A1.

Plasmid pGM1 carries the *E. coli* tryptophan operon containing the deletion ΔLE1413 (G. F. Miozzari, et al., (1978) *J. Bacteriology* 133, 1457–1466)) and hence expresses a fusion protein comprising the first 6 amino acids of the trp leader and approximately the last third of the trp E polypeptide (hereinafter referred to in conjunction as LE'), as well as the trp D polypeptide in its entirety, all under the control of the trp promoter-operator system. The plasmid, 20 μg, was digested with the restriction enzyme PvuII which cleaves the plasmid at five sites. The gene fragments were next combined with EcoRI linkers (consisting of a self complementary oligonucleotide of the sequence: pCATGAATTCATG) (SEQ ID NO:39) providing an EcoRI cleavage site for a later cloning into a plasmid containing an EcoRI site. The 20 μg of DNA fragments obtained from pGM1 were treated with 10 units T_4 DNA ligase in the presence of 200 pico moles of the 5'-phosphorylated synthetic oligonucleotide pCATGAATTCATG and in 20 μl T_4 DNA ligase buffer (20 mM tris, pH 7.6, 0.5 mM ATP, 10 mM $MgCl_2$, 5 mM dithiothreitol) at 4° C. overnight. The solution was then heated 10 minutes at 70° C. to halt ligation. The linkers were cleaved by EcoRI digestion and the fragments, now with EcoRI ends were separated using 5 percent polyacrylamide gel electrophoresis (hereinafter "PAGE") and the three largest fragments isolated from the gel by first staining with ethidium bromide, locating the fragments with ultraviolet light, and cutting from the gel the portions of interest. Each gel fragment, with 300 microliters 0.1×TBE, was placed in a dialysis bag and subjected to electrophoresis at 100 v for one hour in 0.1×TBE buffer (TBE buffer contains: 10.8 gm tris base, 5.5 gm boric acid, 0.09 gm Na_2EDTA in 1 liter H_2O). The aqueous solution was collected from the dialysis bag, phenol extracted, chloroform extracted and made 0.2 M sodium chloride, and the DNA recovered in water after ethanol precipitation. The trp promoter-operator-containing gene with EcoRI sticky ends was identified in the procedure next described, which entails the insertion of fragments into a tetracycline sensitive plasmid which, upon promoter-operator insertion, becomes tetracycline resistant.

Plasmid pBRH1 (R. I. Rodriguez, et al., Nucleic Acids Research 6, 3267–3287 [1979]) expresses ampicilin resistance and contains the gene for tetracycline resistance but, there being no associated promoter, does not express that resistance. The plasmid is accordingly tetracycline sensitive. By introducing a promoter-operator system in the EcoRI site, the plasmid can be made tetracycline resistant.

pBRH1 was digested with EcoRI and the enzyme removed by phenol extraction followed by chloroform extraction and recovered in water after ethanol precipitation. The resulting DNA molecule was, in separate reaction mixtures, combined with each of the three DNA fragments obtained above and ligated with T_4 DNA ligase as previously described. The DNA present in the reaction mixture was used to transform competent *E. coli* K-12 strain 294, K.

Backman et al., Proc Nat'l Acad Sci USA 73, 4174–4198 [1976]) by standard techniques (V. Hershfield et al., Proc Nat'l Acad Sci USA 71, 3455–3459 [1974]) and the bacteria plated on LB plates containing 20 μg/ml ampicillin and 5 μg/ml tetracycline. Several tetracycline-resistant colonies were selected, plasmid DNA isolated and the presence of the desired fragment confirmed by restriction enzyme analysis. The resulting plasmid is designated pBRHtrp.

An EcoRI and BamHI digestion product of the viral genome of hepatitis B was obtained by conventional means and cloned into the EcoRI and BamHI sites of plasmid pGH6 (D. V. Goeddel et al., Nature 281, 544 [1979])) to form the plasmid pHS32. Plasmid pHS32 was cleaved with XbaI, phenol extracted, chloroform extracted and ethanol precipitated. It was then treated with 1 μl *E. coli* polymerase I, Klenow fragment (Boehringer-Mannheim) in 30 μl polymerase buffer (50 mM potassium phosphate pH 7.4, 7 mM $MgCl_2$, 1 mM β-mercaptoethanol) containing 0.1 mM dTTP and 0.1 mM dCTP for 30 minutes at 0° C. then 2 hr. at 37° C. This treatment causes 2 of the 4 nucleotides complementary to the 5' protruding end of the XbaI cleavage site to be filled in. See FIG. **10**.

Two nucleotides, dC and dT, were incorporated giving an end with two 5' protruding nucleotides. This linear residue of plasmid pHS32 (after phenol and chloroform extraction and recovery in water after ethanol precipitation) was cleaved with EcoRI. The large plasmid fragment was separated from the smaller EcoRI-XbaI fragment by PAGE and isolated after electroelution. This DNA fragment from pHS32 (0.2 μg), was ligated, under conditions similar to those described above, to the EcoRI-Taq I fragment of the tryptophan operon (~0.01 μg), derived from pBRHtrp.

In the process of ligating the fragment from pHS32 to the Eco RI-Taq I fragment, as described above, the Taq I protruding end is ligated to the XbaI remaining protruding end even though it is not completely Watson-Crick base-paired. See FIG **11**.

A portion of this ligation reaction mixture was transformed into *E. coli* 294 cells, heat treated and plated on LB plates containing ampicillin. Twenty-four colonies were selected, grown in 3 ml LB media, and plasmid isolated. Six of these were found to have the XbaI site regenerated via *E. coli* catalyzed DNA repair and replication. See FIG. **13**.

These plasmids were also found to cleave both with EcoRI and HpaI and to give the expected restriction fragments. One plasmid, designated pTrp 14, was used for expression of heterologous polypeptides, as next discussed.

The plasmid pHGH 107 (D. V. Goeddel et al, *Nature,* 281, 544, 1979) contains a gene for human growth hormone made up of 23 amino acid codons produced from synthetic DNA fragments and 163 amino acid codons obtained from complementary DNA produced via reverse transcription of human growth hormone messenger RNA. This gene, though it lacks the codons of the "pre" sequence of human growth hormone, does contain an ATG translation initiation codon. The gene was isolated from 10 μg pHGH 107 after treatment with EcoRI followed by *E. coli* polymerase I Klenow fragment and dTTP and dATP as described above. Following phenol and chloroform extraction and ethanol precipitation the plasmid was treated with BamHI.

The human growth hormone ("HGH") gene-containing fragment was isolated by PAGE followed by electroelution. The resulting DNA fragment also contains the first 350 nucleotides of the tetracycline resistance structural gene, but lacks the tetracyline promoter-operator system so that, when subsequently cloned into an expression plasmid, plasmids containing the insert can be located by the restoration of tetracycline resistance. Because the EcoRI end of the fragment has been filled in by the Klenow polymerase I procedure, the fragment has one blunt and one sticky end, ensuring proper orientation when later inserted into an expression plasmid.

The expression plasmid pTrp14 was next prepared to receive the HGH gene-containing fragment prepared above. Thus, pTrp14 was XbaI digested and the resulting sticky ends filled in with the Klenow polymerase I procedure employing dATP, dTTP, dGTP and dCTP. After phenol and chloroform extraction and ethanol precipitation the resulting DNA was treated with BamHI and the resulting large plasmid fragment isolated by PAGE and electroelution. The pTrp14-derived fragment had one blunt and one sticky end, permitting recombination in proper orientation with the HGH gene containing fragment previously described.

The HGH gene fragment and the pTrp14 ΔXba-BamHI fragment were combined and ligated together under conditions similar to those described above. The filled in XbaI and EcoRI ends ligated together by blunt end ligation to recreate both the XbaI and the EcoRI site. See FIG. **13**.

This construction also recreates the tetracycline resistance gene. Since the plasmid pHGH 107 expresses tetracycline resistance from a promoter lying upstream from the HGH gene (the lac promoter), this construction, designated pHGH 207, permits expression of the gene for tetracycline resistance under the control of the tryptophan promoter-operator. Thus the ligation mixture was transformed into *E. coli* 294 and colonies selected on LB plates containing 5 μg/ml tetracycline.

Plasmid pHGH207 was Eco RI digested and the trp promoter containing a 300 b.p. Eco RI fragment recovered by PAGE followed by electroelution. The 300 b.p. Eco RI fragment contains the *E. coli* trp promoter, operator, and trp leader ribosome binding site but lacks an ATG sequence for initiation of translation. This DNA fragment was cloned into the Eco RI site of pLe-IF A. The construction of the fragment is described in detail in (36).

2. The Tryptophan Control Element

The trp fragment just referred to is an analog of the *E. coli* tryptophan operon from which the so-called trp attenuator has been deleted, See (36), to controllably heighten expression levels. Expression plasmids containing the modified trp regulon can be grown to predetermined levels in nutrient media containing additive tryptophan in quantities sufficent to repress the promoter-operator system, then be deprived of tryptophan so as to derepress the system and occasion the expression of the intended product (36).

3. Detailed Description

More particularly, and with reference to FIG. **6**, 250 μg of plasmid pL31 were digested with Pst I and the 1000 b.p. insert isolated by gel electrophoresis on a 6 polyacrylamide gel. Approximately 40 μg of insert was electroeluted from the gel and divided into 3 aliquots for further digestion: a) A 16 μg sample of this fragment was partially digested with 40 units of Bgl II for 45' at 37° C. and the reaction mixture purified on a 6 polyacrylamide gel. Approximately 2 μg of the desired 670 b.p. fragment were recovered. b) Another sample (8 μg) of the 1000 b.p. Pst I insert was restricted with Ava II and Bgl II. One μg of the indicated 150 b.p. fragment was recovered after gel electrophoresis. c) 16 μg of the 1000 b.p. piece was treated with Sau 3a and Ava II. After electrophoresis on a 10 polyacrylamide gel, approximately 0.25 μg (~10 pmole) of the 34 b.p. fragment was recovered. The two indicated deoxyoligonucleotides, 5'-dAATTCATGTGT (SEQ ID NO:42) (fragment 1) and 5'-d GATCACACATG (SEQ ID NO:43) (fragment 2) were

synthesized by the phosphotriester procedure (24). Fragment 2 was phosphorylated as follows. 200 μl (~40 pmole) of (γ^{32}P) ATP (Amersham, 5000 Ci/mmole) was dried down and resuspended in 30 μl of 60 mM Tris-HCl (pH8), 10 mM $MgCl_2$, 15 mM β-merceptoethanol, containing 100 pmoles of DNA fragment and 2 units of T4 polynucleotide kinase. After 15 minutes at 37° C., 1 μl of 10 mM ATP was added and the reaction allowed to proceed another 15 minutes. The mixture was then heated at 70° C. for 15 minutes, combined with 100 pmole of 5'-OH fragment 1 and 10 pmole of the 34 b.p. Sau 3a-Ava II fragment. Ligation was performed for 5 hours at 4° C. in 50 μl of 20 mM Tris-HCl (pH7.5) 10 mM $Mg Cl_2$, 10 mM dithiothreitol, 0.5 mM ATP and 10 units T4 DNA ligase. The mixture was electrophoresed on a 6 polyacrylamide gel and the 45 b.p. product recovered by electroelution. 860,000 Cerenkov cpm were recovered (~30 ng, 1 pmole), combined with 0.5 μg (5 pmoles) of the 150 b.p. Ava II-Bgl II fragment and 1 μg (2 pmoles) of the 670 b.p. Bgl II-Pst I fragment. The ligation was performed at 20° C. for 16 hours using 20 units of T4 DNA ligase. The ligase was inactivated by heating to 65° C. for 10 minutes. The mixture was then digested with Eco RI and Pst I to eliminate polymers of the gene. The mixture was purified by 6 percent polyacrylamide gel electrophoresis. 36,000 cpm (~0.04 pmole, 20 ng) of 865 b.p. product were isolated. One-half (10 ng) of this was ligated into pBR322 (0.3 μg) between the Eco RI and Pst I sites. Transformation of *E. coli* 294 gave 70 tetracycline resistant, ampicillin sensitive tranformants. Plasmid DNA isolated from 18 of these transformants was digested with Eco RI and Pst I. 16 of the 18 plasmids had an Eco RI-Pst I fragment 865 b.p. in length. One μg of one of these, pLe-IF A1, was digested with Eco RI and ligated to a 300 b.p. Eco RI fragment (0.1 μg.) containing the *E. coli* trp promoter and trp leader ribosome binding site, prepared as described above. Transformants containing the trp promoter were identified using a ^{32}P-trp probe in conjunction with the Grunstein-Hogness colony screening procedure (27). An asymetrically located Xba I site in the trp fragment allowed determination of recombinants in which the trp promoter was oriented in the direction of the Le-IF A gene.

K. Induction of Interferon Expression and In Vitro Assay

Extracts were prepared for IF assay as follows. One ml cultures were grown in L broth containing 5 μg/ml tetracycline to an A_{550} of about 1.0, then diluted into 25 ml of M9 media containing 5 μg/ml tetracycline. 10 ml samples were harvested by centrifugation when A_{550} reached 1.0 and cell pellets were suspended in 1 ml of 15 percent sucrose, 50 mM Tris-HCl (pH 8.0), 50 mM EDTA. One mg of lysozyme was added and, after 5 minutes at 0° C., cells were disrupted by sonication. The samples were centrifuged 10 minutes at 15,000 rpm in a Sorvall SM-24 rotor. Interferon activity in the supernatants was determined by comparison with Le-IF standards by the cytopathic effect (CPE) inhibition assay (2). To determine the number of IF molecules per cell a Le-IF specific activity of 4×10^8 units/mg was used (7).

As shown in Table 1, Clone pLe-IF A trp 25, in Which the trp promoter was inserted in the desired orientation, gives high levels of activity (as high as 2.5×10^8 units per liter). As shown in Table 2, the IF produced by *E. coli* K-12 strain 294/pLe-IF A trp 25 behaves like authentic human Le-IF; it is stable to treatment at pH2 and is neutralized by rabbit anti-human leukocyte antibodies. The interferon has an apparent molecular weight of approximately 20,000.

L. In Vivo Antiviral Activity of Le-IF A

The in vivo efficacy of interferon requires the presence of macrophages and NK cells and the in vivo mode of action appears to involve stimulation of these cells (33). Thus, it remained possible that the interferon produced by *E. coli* 294/pLe-IF A25, while having antiviral activity in the cell culture assay, would not be active in infected animals. Moreover, the in vivo antiviral activity of the bacterially produced, non-glycosylated Le-IF A might be different from the glycosylated Le-IF derived from human "buffy coat" leukocytes. Therefore the biological activity of bacterially synthesized Le-IF A (~2 Pure) was compared with buffy coat Le-IF (~8 percent pure) in lethal encephalyomyocarditis (EMC) virus infection of squirrel monkeys (Table 3).

TABLE 1

Interferon activity in extracts of *E. coli*

E. coli K-12 strain 294 transformed by:	Cell density (cells/ml)	IF Activity units/ml culture	Le-IF molecules per cell
pLe-IF A trp 25	3.5×10^8	36,000	9,000
pLe-IF A trp 25	1.8×10^9	250,000	12,000

TABLE 2

Comparison of activities of extracts from *E. coli* 294/pLe-IF A25 with standard Le-IF

	Interferon Activity (units/ml)		
	untreated	pH2	rabbit anti-human leukocyte antibodies
294/pLeIF-A trp 25 extract	500	500	<10
Le-IF standard	500	500	<10

TABLE 3

Antiviral effect of various Le-IF preparations against EMC virus infection of squirrel monkeys

Treatment	Survivors	Serum p.f.u./ml. day 2		Serum p.f.u./ml. day 3		Serum p.f.u./ml. day 4	
Control (bacterial proteins)	0/3	10	3	3×10^4	10^4	$>10^5$	$>3.4\times10_4$
		0		0		1,200	
		0		0		0	
Bacterial Le-IF A	3/3	0		0		0	
		0		0		0	
		0		0		0	
Le-IF standard	3/3	0		0		0	
		0		0		0	
		0		0		0	

All monkeys were male (average weight 713 g) and had no EMC virus antibodies prior to infection. The monkeys were infected intramuscularly with $100\times LD_{50}$ EMC virus (determined in mice). The control treated monkeys died at 134, 158 and 164 hours post-infection. Interferon treatments of 10^6 units were by the intravenous route at −4, +2, 23, 29, 48, 72, 168 and 240 hours, relative to infection. The bacterial leukocyte interferon was a column chromatography fraction from a lysate of *E. coli* 294/pLe-IF A25 at a specific activity of 7.4×10^6 U/mg protein. The control bacterial proteins were an equivalent column fraction from a lysate of *E. coli* 294/pBR322 at twice the total protein concentration. The leukocyte interferon standard was Sendai virus induced interferon from normal human "buffy-coat" cells, purified chromatographically to a specific activity of 32×10^6 U/mg protein.

The control monkeys showed progressive lethargy, loss of balance, flaccid paralysis of the hind-limbs and watering of the eyes commencing around 8 hours prior to death. The interferon treated monkeys showed none of these abnormalities; they remained active at all times and developed no viremia (Table 3). The one monkey in the control group which did not develop viremia by 4 days died latest (164 h post-infection) but showed high titers of virus in the heart and brain on post-mortem. The interferon treated monkeys did not develop antibodies to EMC virus as determined 14 and 21 days after infection. These results demonstrate that the antiviral effects of Le-IF preparations in the infected animals can be attributed solely to interferon because the contaminating proteins are quite different in the bacterial and buffy coat preparations.

M. Isolation of cDNAs for Additional Leukocyte Interferons

DNA from the fully characterized Le-IF A cDNA-containing plasmid was excised with Pst I, isolated electrophoretically, and labelled by a published procedure (26) with ^{32}P. The resulting radioactively labelled DNA was used as a probe to screen-additional *E. coli* 294 transformants, obtained identically as those in Part D, by an in situ colony screening procedure (27). Colonies were isolated which hybridized in varying amounts to the probe. Plasmid DNA from these colonies and the ten hybridizing colonies referred to in Part I above was isolated by Pst cutting and characterized by three different methods. First, these Pst fragments were characterized by their restriction endonuclease digestion patterns with the enzymes Bgl II, Pvu II, and Eco RI. This analysis allowed the classification of at least eight different types (Le-IF A, Le-IF B, Le-IF C, Le-IF D, Le-IF E, Le-IF F, Le-IF G and Le-IF H), shown in FIG. **5**, which approximates the location of various restriction cuts relative to the by-now known presequence and coding sequence of Le-IF A. One of these, Le-IF D, is believed to be identical to that reported in (39).

Secondly, certain of the DNAs were tested by a published hybridization selection assay (38) for the ability to selectively remove Le-IF mRNA from poly-A containing KG-1 cell RNA. Le-IF A, B, C and F were positive by this assay. Third, the latter Pst fragments were inserted in an expression plasmid, *E. coli* 294 transformed with the plasmid, and the fragments expressed. The expression products, believed to have been preinterferons, were all positive by CPE assay for interferon activity, albeit marginally active in the case of the Le-IF-F fragment. In addition to the foregoing, all of the Le-IF types described have been sequenced (See FIG. **3**).

N. Direct Expression of a Second Mature Leukocyte Interferon

The sequence of the isolated fragment comprising the gene for mature Le-IF-B shows the first fourteen nucleotides of types A and B to be identical. We accordingly proposed to isolate a fragment from pLe-IF A25 bearing the trp-promoter-operator, ribosome binding site and the start of the Le-IF (A=B) gene, and combine this with the remaining portion of the B sequence in an expression plasmid. The salient restriction maps for the Pst fragment of pL4 (a plasmid comprising the Le-IF B Pst-ended gene depicted in FIG. **5**) and pLe-IF A25 are shown, respectively, in FIGS. **7***a* and **7***b*.

To obtain the approximately 950 b.p. Sau 3a to Pst I fragment from the sequence shown in FIG. **7***a* several steps were necessary because of the presence of one or more intervening Sau 3a restriction sites, i.e.:

1. The following fragments were isolated:

a) 110b b.p. from Sau 3a to Eco RI;

b) 132 b.p. from Eco RI to Xba;

c) >700 b.p. from Xba to Pst.

2. Fragments (1a) and (1b) were ligated and cut with Xba and Bgl II to preclude self-polymerization through Sau 3a and Xba end terminals (the relevant Sau 3a site was within a Bgl II site; Bgl II cuts to leave a Sau 3a sticky end). A 242 b.p. fragment was isolated.

3. The product of (2) and (1c) were ligated and cut with Pst I and Bgl II, again to prevent self-polymerization. An approximate 950 b.p. fragment, Sau 3a to Pst I of FIG. **7***a*, was isolated. This fragment comprised that portion of the Le-IF B gene not common to Le-IF A.

4. An approximate 300 b.p. fragment (Hind III to Sau 3a) comprising the trp promoter-operator, ribosome binding site, ATG start signal and cysteine codon of Le-IF A was isolated from pLe-IF A25.

5. An approximately 3600 b.p. fragment Pst I to Hind III was isolated from pBr 322. This comprised the replicon and encoded tetracycline but not ampicillin resistance.

6. The fragments obtained in steps 3, 4 and 5 were triple-ligated and the resulting plasmid transformed into *E. coli* K-12 strain 294.

Transformants were miniscreened (37) and plasmid samples were digested with Eco RI. Digests yielded three fragments characteristic of:

1) The Eco RI-Eco RI trp promoter fragment; 2) The internal Eco RI to Eco RI fragment of pL4; and 3) protein translational start signal to Eco RI fragment of pL4.

In CPE assay, bacterial extracts from clones made in the foregoing fashion typically assay at about 10×10^6 units interferon activity per liter at A_{550}=1. One representative clone prepared in this manner is 294/pLIF B trp 7.

O. Direct Expression of Further Mature Leukocyte Interferons

Additional full-length gene fragments that comprise other Le-IF types may be tailored and placed in expression vehicles for expression as in the case of Le-IF A. Complete sequencing by conventional means will reveal whether a restriction site lies sufficiently near the first amino acid codon of the mature interferon type as to permit convenient resort to the approach employed in part J, supra, for the expression of mature Le-IF A, i.e., elimination of the presequence by restriction cutting and replacement of codons for the N-terminal amino acids lost in presequence elimination by ligation of a synthetic DNA fragment; Failing that, the procedure described in (36) may be employed. Briefly, this entails cleaving the presequence-containing fragment precisely before the point at which the codon for the first amino acid of the mature polypeptide begins, by:

1. converting the double stranded DNA to single-stranded DNA in a region surrounding that point;
2. hybridizing to the single-stranded region formed in step (a) a complementary primer length of single-stranded DNA, the 5' end of the primer lying opposite the nucleotide adjoining the intended cleavage site;
3. restoring that portion of the second strand eliminated in step 1 which lies in the 3' direction from the primer by reaction with DNA polymerase in the presence of adenine, thymine, guanine and cytosine-containing deoxynucleotide triphosphates; and
4. digesting the remaining single-stranded length of DNA which protrudes beyond the intended cleavage point.

A short length of synthetic DNA terminating, at the 3' end of the coding strand, with the translation start signal ATG can then be ligated by, e.g., blunt-end ligation to the resulting tailored gene for the mature interferons and the gene inserted into an expression plasmid and brought under the control of a promoter and its associated ribosome binding site.

In a manner similar to that employed in part N; supra, gene fragments encoding Le-IF-C and Le-IF-D were appropriately configured for direct bacterial expression. The expression strategy for these additional leukocyte interferons included, in each case, resort to the approximately 300 b.p. fragment (Hind III to Sau 3a) comprising the trp promoter-operator, ribosome binding site, ATG start signal and cysteine codon of Le-IF A from pLe-IF A25. To this were combined gene fragments from the additional interferon genes encoding their respective amino acid sequences beyond the initial cysteine common to all. Each resulting plasmid was used to transform *E. coli* K-12 strain 294. Ligations to form the respective genes were as follows:

Le IF-C

Isolate the following fragments from pLe IF-C:

(a) 35 b.p. from Sau 3a to Sau 96

(b) >900 b.p. Sau 96 to Pst

(c) Isolate an approximate 300 b.p. fragment (Hind III-Sau 3a) from pLe IF A-25 as in part N (4) supra.

(d) Isolate the approximately 3600 b.p. fragment of part N (5) supra.

Construction

(1) Ligate (a) and (c). Cleave with Bgl II, Hind III and isolate the approximately 335 b.p. product.

(2) Triple ligate (1)+(b)+(d) and transform *E. coli* with the resulting plasmid.

A representative clone made in this manner is *E. coli* K-12 strain 294/pLe IF C trp 35.

Le-IF D

Isolate from pLe IF-D:

a) 35 b.p. from Sau 3a to Ava II

b) 150 b.p. from Ava II to Bgl II

c) approx. 700 b.p. from Bgl II to Pst

Isolate from pLe IF A25:

d) 300 b.p. from Hind III to Sau 3a

Isolate from PBr 322:

e) approx. 3600 b.p. from Hind III to Pst

Construction

(1) ligate (a)+(b), cut with Bgl II and purify a 185 b.p. product (1).

(2) ligate (1)+(d), cut with Hind III, Bgl II, and purify the approx. 500 b.p. product (2).

(3) ligate (2)+(c)+(e) and transform *E. coli* with the resulting plasmid.

A representative clone made in this manner is *E. coli* K-12 strain 294/pLeIF D trp 11.

Le-IF F

The Le-IF F containing fragment may be tailored for direct expression through reassembly made convenient by the complete homology of amino acids 1–13 of Le-IF B and Le-IF F. A trp promoter-containing fragment (a) with appropriately configured ends is obtained from pHGH 207, described above, via Pst I and Xba I digestion followed by isolation of the ca. 1050 b.p. fragment. A second fragment (b) is obtained as the larger of the fragments resulting from Pst I and Bgl II digestion of the plasmid pHKY 10 (36). Fragment (a) contains approximately half the gene encoding amplicillin resistance; fragment (b) contains the remainder of that gene and the entire gene for tetracycline resistance save for the associated promoter. Fragments (a) and (b) are combined via T4 ligase and the product treated with Xba I and Bgl II to eliminate dimerization, forming a fragment (c) comprising the trp promoter-operator and genes for tetracycline and ampicillin resistance.

A fragment (d) of approximately 580 b.p. is obtained by Ava II and Bgl II digestion of pLe IF-F. This comprises codons for amino acids 14–166 of Le-IF F.

A fragment (e) (49 b.p.) is obtained by Xba I and Ava II digestion of pLe-IF B. Fragment (e) encodes amino acids 1–13 of Le-IF F.

Fragments (c), (d) and (e) are triple ligated in the presence of T4 ligase. The cohesive ends of the respective fragments are such that the composite plasmid circularizes correctly, bringing the tetracycline resistance gene under the control of the trp promoter-operator along with the gene for mature Le-IF F, such that bacteria transformed with the desired plasmid may be selected on tetracycline-containing plates. A representative clone prepared in this manner is *E. coli* K-12 strain 294/pLeIF F trp 1.

Le-IF H

The complete Le-IF H gene may be configured for expression as a mature leukocyte interferon as follows:

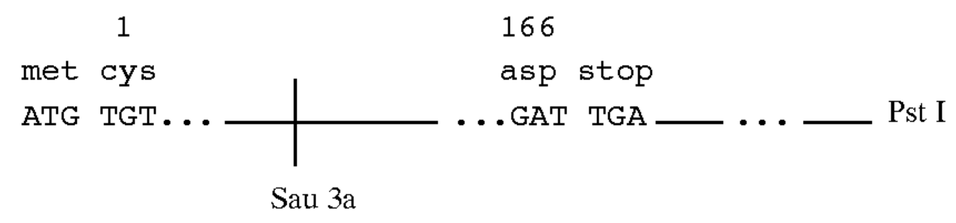
1. Plasmid pLe-IF H is subjected to Hae II and Rsa I digestion with isolation of the 816 base pair fragment extending from the signal peptide amino acid 10 to the 3' noncoding region.

2. The fragment is denatured and subjected to repair synthesis with Klenow fragment, Klenow et al., *Proc. Natl. Acad. Sci.* (*USA*) 65, 168 (1970), employing the synthetic deoxyribooligonucletide primer 5'-dATG TGT AAT CTG TCT. This general procedure is also de scribed by Goeddel et al., U.S. Ser. No. 190799, filed Sep. 25, 1980.

3. The resulting product is cleaved with Sau 3a and a 452 base pair ("bp") fragment representing amino acids 1 to 150 isolated.

4. Sau 3a and Pst I digestion of pLeIF H and isolation of the resulting 500 b.p. fragment yields a gene encoding amino acids 150 through the end of the coding sequence.

5. Fragments isolated in steps (3) and (4) are ligated to form a fragment:



encoding the 166 amino acids of Le-IF H.

6. pLeIF A trp 25 is digested with Xba I, blunt-ended with DNA polymerase I and the product digested with Pst I. The large resulting fragment may be isolated and ligated with the product of step (5) to form an expression plasmid capable, upon transformation of *E. Coli* K-12 strain 294 or other host bacteria, of expressing mature Le-IF H.

LeIF-I

The phage λ Charon 4A recombinant library of the human genome constructed by Lawn et al., *Cell* 15, 1157 (1978), was screened for leukocyte interferon genes by procedures described by Lawn et al., Supra and Maniatis et al., *Cell* 15, 687 (1978). A radioactive LeIF probe derived from the cDNA clone LeIF A (Goeddel et al., *Nature* 287, 411 (1980), was used to screen approximately 500,000 plaques. Six LeIF genome clones were obtained in this screening. Following rescreening and plaque purification, one of these clones, λHLeIF2, was selected for further analysis.

Using the method described above, other probes can be used to advantage to isolate additional LeIF clones from the human genome. These, in turn, can be employed to produce additional leukocyte interferon proteins in accordance with this invention.

1. The 2000 base pair Eco RI fragment of the genomic clone (λHLeIF2) was subcloned into pBR325 at the Eco RI site. The resulting plasmid LeIF I was cleaved with Eco RI and the 2000 base pair fragment isolated. The deoxyoligonucleotide dAATTCTGCAG (SEQ ID NO:44) (an Eco RI>Pst I convertor) was ligated to the 2000 base pair Eco RI fragment and the resulting product cleaved with Pst I to give a 2000 base pair fragment containing Pst I ends. This was cleaved wtih Sau 96 and a 1100 base pair fragment isolated which has one Pst I end and one Sau 96 end.

2. The plasmid pLeIF C trp 35 was digested with Pst I and Xba I. The large fragment was isolated.

3. The small Xba I-Pst I fragment from pLeIF C trp 35 was digested with Xba I and Sau 96. A 40 base pair Xba I-Sau 96 fragment was isolated.

4. The fragments isolated in steps 1), 2) and 3) were ligated to form the expression plasmid pLeIF I trp 1.

LeIF-J

1. The plasmid pLeIF J contains a 3.8 kilobase Hind III fragment of human genomic DNA which includes the LeIF J gene sequence. A 760 base pair Dde I-Rsa I fragment was isolated from this plasmid.

2. The plasmid pLeIF B trp 7 was cleaved with Hind III and Dde I and a 340 bp Hind III-Dde I fragment isolated.

3. The plasmid pBR322 was cleaved with Pst I, blunt ended by incubation with DNA Pol I (Klenow fragment), then digested with Hind III. The large (~3600 bp) fragment was isolated.

4. Fragments isolated in steps 1), 2) and 3) were ligated to form the expression plasmid pLeIF J trp 1.

P. Purification

The content of leukocyte interferon in bacterial extracts may be enhanced by successive:

1. polyethylene-imine precipitation, in which most of the cellular protein, including the interferon, remains in the supernatant;
2. ammonium sulfate fractionation, in which interferon comes out of solution in 55 saturated ammonium sulfate;
3. suspension of the ammonium sulfate pellet in 0.06M potassium phosphalte, 10 mM tris-HCl, pH 7.2, and dialysis against 25 mM tris-HCl, pH 7.9 (interferon activity remains in solution); and
4. Loading the above supernatant, pH adjusted to 8.5, on a DEAE-cellulose (Whatman DE-53) column and eluting with a linear gradient of 0 to 0.2M NaCl in 25 mM tris HCl, pH 8.5.
5. Adsorption on Cibachrome Blue Agarose (Amicon Blue A) or hydroxyapatitie and elution with high salt (1.5 M KCl or 0.2 M phosphate respectively)—optional.
6. Molecular sizing on a Sephadex G-75 column.
7. Cation exchange chromatography on CM-cellulose (Whatman CM-52) in 25 mM ammonium acetate at pH 5.0, developed with an ammonium acetate gradient (to 0.2 M ammonium acetate).

In our hands, the above process gives essentially homogeneous material (e.g. >95 percent pure).

The material can also be further purified by further steps such as, in succession:

8. Size exclusion chromatography;
9. Reverse phase (RP-8) high pressure liquid chromatography; and if desired
10. Affinity chromatography on immobilized antiinterferon antibodies.

Affinity chromatography on a monoclonal antibody column can be used as an alternative to the Step 6 Sephadex G-75 column above. The material from step 4 is loaded on the monoclonal antibody column, prepared as described by Milstein, C., *Scientific American* 243, No. 4, p. 66 (1980), and eluted with 0.2 M acetic acid, 0.1 percent tritqn and 0.15 M NaCl.

In an alternative, preferred embodiment, the leukocyte interferon produced by the procedures described herein can be purified by the following steps:

1. Frozen cell pellets containing the expressed leukocyte interferon are broken up manually or by appropriate size reduction equipment. The partially thawed cells are suspended in 4 volumes of buffer A. The suspension is held to approximately 4° C.

Buffer A:

0.1 M Tris adjusted to pH 7.5–8.0
10% (w/v) sucrose
0.2 M NaCl
5 mM EDTA
0.1 mM PMSF
10–100 mM $MgCl_2$

The suspension is passed through a Manton Gaulin laboratory homogenizer at about 6000 psi followed by a second pass at less than 1000 psi. Effluent from the homogenizer from both passes is cooled in an ice bath.

2. Polyethylene-imine (PEI) (e.g. Polymin P) is added slowly to the homogenate to a concentration of about 0.35% and allowed to stand for about 30 min. The solids are removed by centrifugation or filtration. This step is temperature controlled or performed sufficiently quickly that the supernatant (filtrate) is kept at less than 10° C. The supernatant (filtrate) is concentrated by ultrafiltration, e.g. on a Millipore Pellicon cassette system (PTGC, 5 $ft.^2$, MWCO 10,000), to approximately 1/10 the original volume. Particulate matter or haziness in the retenate may be removed by an appropriate filter such as a microporous membrane.

3. The clarified solution is loaded directly onto a monoclonal antibody column at a flux of 5–8 cm/hr. (e.g. 25–40 ml/hr. on 2.6 cm Diam column). After loading the column is washed with approximately 10 column volumes of 25 mM Tris HCl, pH 7.5–8.5 including NaCl (0.5M) and surfactant such as Triton X-100 (0.2%) or equivalent. Following the wash the column is rinsed with about 10 column volumes of solution containing 0.15 M NaCl and surfactant such as Triton X-100 (0.1%) or equivalent. The column is eluted with 0.2 M acetic acid containing surfactant such as Triton X-100 (0.1%) or equivalent. The protein peak from the monoclonal antibody column (as determined by UV absorbence or other convenient assay) is pooled and the pH adjusted to approximately 4.5 with 1 N NaOH or 1.0 M Tris base.

4. The pooled interferon peak is loaded onto a cationic exchanger such as Whatman CM52 cellulose or equivalent which has been equilibrated with suitable buffer such as ammonium acetate pH 4.5 (50 mM). After loading, the column is washed with equilibrating buffer until the UV absorbence of the effluent has reached a plateau so that little additional protein is eluting from the column. The column is then eluted with 25 mM ammonium acetate/0.12 M sodium

chloride or a combination which optimizes recovery of interferon and affords a lyophilized cake having satisfactory appearance and solubility properties.

The monoclonal antibodies employed in the preferred embodiment described above can be prepared by the procedures described by Staehelin, et. al., *P.N.A.S.,* 78, pp. 1848–52 (1981). Monoclonal antibodies are purified and covalently linked to Affigel-10 as described below:

Preparation and Purification of Monoclonal antibodies from Ascitic Fluid

Five female Balb/c mice were each inoculated with 5 to 10×10^6 hybridoma cells from mid-log growth phase. About 5×10^6 viable cells obtained from the mouse producing fluid were inoculated intraperitoneally into each of 10 or more mice. The ascitic fluid was collected repeatedly (2 to 4 times) from each mouse. Up to three transfers and collections may be performed from one group of mice to the next. Ascitic fluid from mice at each transfer was pooled.

Cells and debris were removed from the ascitic fluid by low speed centrifugation (500–1000×g) for 15 min. Then centrifugation was performed for 90 min. at 18,000 rpm in the SS34 Sorvall rotor without braking. The supernatant was frozen and stored at −20° C. After thawing, additional fibrin and particulate material were removed by centrifugation at 35,000 rpm for 90 min. in the Type 35 Spinco rotor. Batches of ascitic fluid from each transfer were tested for specific antibody activity by a solid phase antibody-binding assay (Staehelin, et. al., *P.N.A.S.,* 78, pp. 1848–52 (1981) and pooled if found satisfactory.

Concentration of protein in the pooled solutions was estimated by the approximation that 1 mg of protein yields an absorbance of 1.2 at 280 nm in a cuvette with a path length of 1.0 cm. Ascites fluids with high levels of antibody contain 30 to 35 mg protein/ml. This is equivalent to 4–7 mg of specific antibody/ml. The fluid was diluted with PBS (0.01 M sodium phosphate, pH 7.3, 0.15 M NaCl) to a protein concentration of 10 to 12 mg/ml (12 to 15 A_{280} units/ml).

To each 100 ml of diluted solution, 90 ml of room temperature saturated ammonium sulfate solution was added slowly with vigorous stirring at 0° C. The suspension was kept in ice for 40 to 60 min., then centrifuged for 15 min. at 10,000 rpm in a Sorvall GS-A rotor at 4° C. The supernatant was decanted and drained well. The protein pellets were dissolved in 0.02 M Tris.HCl (pH 7.9)/0.04 M NaCl (Buffer A; about 5 ml per 250 ml centrifuge bottle). The protein solution was dialyzed for 16 to 18 hrs. at room temperature against 100 volumes of Buffer A with at least one change of the buffer. The dialyzed solution was centrifuged at 15,000 rpm in a SS34 Sorvall rotor for 10 min. to remove undissolved material. About 30% to 35% of the original amount of total protein in the ascitic fluid was recovered as estimated by absorption at 280 nm.

The solution containing 30 to 40 mg of protein per ml was then applied to a column of DEAE-cellulose (DE52, Whatman) equilibrated with Buffer A. A column bed volume of at least 100 ml was used for each gram of protein applied. The antibody was eluted from the column with a linear NaCl gradient containing 0.02 M Tris.HCl, pH 7.9, from 0.04 M to 0.5 M NaCl. Pooled peak fractions eluting between 0.06 and 0.1 M NaCl were concentrated by precipitation with an equal volume of room temperature saturated ammonium sulfate and centrifugation. The protein pellets were dissolved in 0.2 M $NaHCO_3$ (pH ~8.0)/0.3 M NaCl (Buffer B) followed by dialysis against three changes of the same buffer at room temperature. The dialyzed solutions were centrifuged at 20,000×g for 15 min. to remove any insoluble material. Protein concentration was adjusted to 20 to 25 mg/ml with Buffer B. SDS-polyacrylamide gel electrophoresis of representative monoclonal antibodies is shown in FIG. **1**.

Preparation of Immunoadsorbants

Affigel-10 (BioRad Laboratories, Richmond, Calif.) was washed on a sintered glass filter three times with ice-cold isopropanol followed by three washes with ice-cold distilled water. The gel slurry (~50% in cold water) was transferred to plastic tubes and sedimented by a brief centrifugation. The supernatant was aspirated. The packed gel was mixed with an equal volume of purified antibody solution and rotated end-over-end at 4° C. for 5 hrs. After reaction, the gel was centrifuged, then washed twice with Buffer C (0.1 M $NaHCO_3$/0.15 M NaCl) to remove uncoupled antibody. Protein determination of the combined washes revealed that more than 90% of antibody was coupled to the gel.

To block unreacted sites, the gel was mixed with an equal volume of 0.1 M ethanolamine.HCl (pH 8) and rotated end-over-end at room temperature for 60 min. The gel slurry was washed free of reactants with PBS and stored in PBS in the presence of 0.02% (w/v) sodium azide at 4° C.

Q. Parenteral Administration

Le-IF may be parenterally administered to subjects requiring antitumor, or antiviral treatment, and to those exhibiting immunosuppressive conditions. Dosage and dose rats may parallel that currently in use in clinical investigations of human derived materials, e.g., about $(1–10)\times10^6$ units daily, and in the case of materials of purity greater than 1 percent, likely up to, e.g., 50×10^6 units daily. Preliminary indications in the monkey study described above suggest that dosages of bacterially obtained Le-IF could be significantly elevated for greater effect owing to the essential absence of human proteins other than Le-IF, which proteins in leukocyte-derived materials may act as pyrogens, exhibiting adverse effects, e.g., malaise, temperature elevation, etc.

As one example of an appropriate dosage form for essentially homogeneous bacterial Le-IF in parenteral form, 3 mg. Le-IF of specific activity of, say, 2×10^8 U/mg may be dissolved in 25 ml. 5 N serum albumin (human)—USP, the solution passed through a bacteriological filter and the filtered solution aseptically subdivided into 100 vials, each containing 6×10^6 units pure interferon suitable for parenteral administration. The vials are preferably stored in the cold (−20° C.) prior to use.

The compounds of the present invention can be formulated according to known methods to prepare pharmaceutically useful compositions, whereby the polypeptide hereof is combined in admixture with a pharmaceutically acceptable carrier vehicle. Suitable vehicles and their formulation are described in Remington's *Pharmaceutical Sciences* by E. W. Martin, which is hereby incorporated by reference. Such compositions will contain an effective amount of the interferon protein hereof together with a suitable amount of vehicle in order to prepare pharmaceutically acceptable compositions suitable for effective administration to the host. One preferred mode of administration is parenteral.

REFERENCES

1. Isaacs, A. and Lindenmann, J. *Proc. R. Soc.* B147, 258–267 (1957).
2. Stewart, W. E. II *The Interferon System,* Springer, N.Y. (1979).
3. U.S. Pat. No. 3,699,222.
4. U.S. patent application Ser. No. 106,644 filed Dec. 26, 1979 by S. Pestka and M. Rubenstein, assigned to Hoffman LaRoche, Inc.

5. K. Itakura et. al, *Science* 198, 1056 (1971).
6. Cavalieri, R. L., Havell, E. A., Vileck, J. and Pestka, S. *Proc. Natn. Acad. Sci. U.S.A.* 74, 3287–3291 (1977).
7. Rubinstein, M., Rubinstein, S., Familletti, P. C., Miller, R. S., Waldman, A. A. and Pestka, S. *Proc. Natn. Acad. Sci. U.S.A.* 76, 640–644 (1979).
8. Zoon, K. C., Smith, M. E., Bridgen, P. J., zur Nedden, D. and Anfinsen, C. B. *Proc. Natn. Acad. Sci. U.S.A.* 76, 5601–5605 (1979).
9. D. V. Goeddel et. al, *Proc. National Academy of Sciences,* USA 76, 106 (1979).
10. G. B. Patent Publication No. 2 007 676 A.
11. U.S. Pat. No. 4,190,495.
12. British Patent Publication No. 2055382A.
13. (No reference)
14. Chirgwin, J. M. et. al (1979) *Biochemistry* 18, 5294.
15. Koeffler, H. P. and Golde, D. W. *Science* 200, 1153–1154 (1978).
16. Green, M. et. al (1976) *Arch. Biochem. Biophys.* 172, 74–89.
17. Wickens, M. P., Buell, G. N. and Schimke, R. T. *J. Biol. Chem.* 253, 2483–2495 (1978).
18. Goeddel, D. V., Heyneker, H. L., Hozumi, T., Arentzen, R., Itakura, K., Yansura, D. G., Ross, M. J., Miozzari, G., Crea, R. and Seeburg, P. H. *Nature* 281, 544–548 (1979).
19. Chang, A. C. Y., Nunberg, J. H., Kaufman, R. J., Erlich, H. A, Schimke, R. T. and Cohen, S. N. *Nature* 275, 617–624 (1978).
20. Bolivar, F., Rodriguez, R. L., Greene, P. J., Betlach, M. C., Heyneker, H. L., Boyer, H. W., Crosa, J. H. and Falkow, S. *Gene* 2, 95–113 (1977).
21. Okuyuma, A. et. al (1978) *Arch. Biochem. Biophys.* 188, 98.
22. Birnboim., H. C. and Doly, J. *Nucleic Acids Res.* 7, 1513–1523 (1979).
23. Kafatos, F. C., Jones, C. W. and Efstratiadis, A: *Nucleic Acids Res.* 7, 1541–1552 (1979).
24. Crea, R., Kraszewski, A., Hirose, T. and Itakura, K. *Proc. Natn. Acad. Sci. U.S.A.* 75, 5765–5769 (1978).
25. Noyes, B., Mevarech, M., Stein, R. and Agarwal, K. L. *Proc. Natn. Acad. Sci. U.S.A.* 76, 1770–1774 (1979).
26. Taylor, J. M., Illemensee, R. and Summers, S. *Biochim. Biophys. Acta* 442, 324–330 (1976).
27. Grunstein, M. and Hogness, D. S., *Proc. Natn. Acad. Sci. U.S.A.* 72, 3961–3965 (1975).
28. Wallace, R. B., Shaffer, J., Murphy, R. F., Bonner, J. and Itakura, K. *Nucleic Acids Res.* 6, 3543–3557 (1979).
29. Clewell, D. B. and Helinski, D. R. *Biochemistry* 9, 4428–4440 (1970).
30. Maxam, A. M. and Gilbert, W. *Methods Enzymol.* 65, 499–560 (1980).
31. Smith, A. J. H. *Methods Enzymol.* 65, 560–580 (1980).
32. Lillenhaug, J. R. et. al (1976) *Biochemistry* 15, 1858
33. Herberman, R. B., Djeu, J. V., Ortaldo, J. R., Holen., H. T., West, W. H. and Bonnard, G. D. *Cancer Treat. Rep.* 62, 1893–1896 (1978); Gidlund, M., Orn, A., Wigzell, H., Senik, A. and Gressor I. *Nature* 273, 759–761 (1978); Stebbing, N., Dawson, K. M. and Lindley, I. J. D. *Infect. Immun.* 19, 5–11 (1978).
34. Clewell, D. B. (1970) *Biochemistry* 9, 4428.
35. Radloff, R. et. al (1967) *Proc. Natl. Acad. Sci. U.S.A.* 57, 1514. 136.
36. U.S. patent application Ser. No. 06/133,296 filed Mar. 24, 1980 by Dennis G. Kleid et. al, assigned to Genentech, Inc.
37. Birnboim, H. C. and Doly, J., *Nucleic Acids Research* 7, 1513 (1979)
38. Cleveland, D. W. et. al (1980) *Cell* 20, 95
39. Nagata, S. et. al, *Nature* 284, 316 (1980)

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Leu Phe Ser Cys Leu Lys Asp Arg His Asp Phe Gly Phe Pro Gln Glu
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Glu Met Ile Gln Gln Ile Phe Asn Leu Phe Ser Thr Lys Asp Ser Ser
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Ala Ala Trp Asp Glu Thr Leu Leu Asp Lys Phe Tyr Thr Glu Leu Tyr
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 85                  90                  95                  100

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165                 170                 175                 180

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Pro Phe Ser Cys Leu Lys Asp Arg His Asp Phe Arg Ile Pro Gln Glu
    50                  55                  60

Glu Phe Asp Gly Asn Gln Phe Gln Lys Ala Gln Ala Ile Ser Val Leu
65                  70                  75                  80

His Glu Met Ile Gln Gln Thr Phe Asn Leu Phe Ser Thr Glu Asp Ser
                85                  90                  95

Ser Ala Ala Trp Glu Gln Ser Leu Leu Glu Lys Phe Ser Thr Glu Leu
            100                 105                 110

Tyr Gln Gln Leu Asn Asp Leu Glu Ala Cys Val Ile Gln Glu Val Gly
        115                 120                 125

Val Glu Glu Thr Pro Leu Met Asn Glu Asp Ser Ile Leu Ala Val Arg
```

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```
    130                 135                 140

Lys Tyr Phe Gln Arg Ile Thr Leu Tyr Leu Ile Glu Arg Lys Tyr Ser
145                 150                 155                 160

Pro Cys Ala Trp Glu Val Val Arg Ala Glu Ile Met Arg Ser Leu Ser
                165                 170                 175

Phe Ser Thr Asn Leu Gln Lys Arg Leu Arg Arg Lys Asp
            180                 185
```

<210> SEQ ID NO 7
<211> LENGTH: 863
<212> TYPE: DNA
<213> ORGANISM: Homo sapiens
<220> FEATURE:
<221> NAME/KEY: CDS
<222> LOCATION: (55)...(621)

<400> SEQUENCE: 7

```
caaggttcag agtcacccat ctcagcaagc ccagaagtat ctgcaatatg tacg atg       57
                                                           Met
                                                            1

gcc tcg ccc ttt gct tta ctg atg gtc ctg gtg gtg ctc agc tgc aag      105
Ala Ser Pro Phe Ala Leu Leu Met Val Leu Val Val Leu Ser Cys Lys
            5                  10                  15

tca agc tgc tct ctg ggc tgt gat ctg cct gag acc cac agc ctg gat      153
Ser Ser Cys Ser Leu Gly Cys Asp Leu Pro Glu Thr His Ser Leu Asp
        20                  25                  30

aac agg agg acc ttg atg ctc ctg gca caa atg agc aga atc tct cct      201
Asn Arg Arg Thr Leu Met Leu Leu Ala Gln Met Ser Arg Ile Ser Pro
    35                  40                  45

tcc tcc tgt ctg atg gac aga cat gac ttt gga ttt ccc cag gag gag      249
Ser Ser Cys Leu Met Asp Arg His Asp Phe Gly Phe Pro Gln Glu Glu
50                  55                  60                  65

ttt gat ggc aac cag ttc cag aag gct cca gcc atc tct gtc ctc cat      297
Phe Asp Gly Asn Gln Phe Gln Lys Ala Pro Ala Ile Ser Val Leu His
                70                  75                  80

gag ctg atc cag cag atc ttc aac ctc ttt acc aca aaa gat tca tct      345
Glu Leu Ile Gln Gln Ile Phe Asn Leu Phe Thr Thr Lys Asp Ser Ser
            85                  90                  95

gct gct tgg gat gag gac ctc cta gac aaa ttc tgc acc gaa ctc tac      393
Ala Ala Trp Asp Glu Asp Leu Leu Asp Lys Phe Cys Thr Glu Leu Tyr
        100                 105                 110

cag cag ctg aat gac ttg gaa gcc tgt gtg atg cag gag gag agg gtg      441
Gln Gln Leu Asn Asp Leu Glu Ala Cys Val Met Gln Glu Glu Arg Val
    115                 120                 125

gga gaa act ccc ctg atg aat gtg gac tcc atc ttg gct gtg aag aaa      489
Gly Glu Thr Pro Leu Met Asn Val Asp Ser Ile Leu Ala Val Lys Lys
130                 135                 140                 145

tac ttc cga aga atc act ctc tat ctg aca gag aag aaa tac agc cct      537
Tyr Phe Arg Arg Ile Thr Leu Tyr Leu Thr Glu Lys Lys Tyr Ser Pro
                150                 155                 160

tgt gcc tgg gag gtt gtc aga gca gaa atc atg aga tcc ctc tct tta      585
Cys Ala Trp Glu Val Val Arg Ala Glu Ile Met Arg Ser Leu Ser Leu
            165                 170                 175

tca aca aac ttg caa gaa aga tta agg agg aag gaa taatatctgg           631
Ser Thr Asn Leu Gln Glu Arg Leu Arg Arg Lys Glu
        180                 185

tccaacatga aaacaattct tattgactca tacaccaggt cacgctttca tgaattctgt    691

catttcaaag actctcaccc ctgctataac tatgaccatg ctgataaact gatttatcta    751

tttaaatatt tatttaacta ttcataagat ttaaattatt tttgttcata taacgtcatg    811
```

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tgcaccttta cactgtggtt agtgtaataa aacatgttcc ttatatttac tc 863

<210> SEQ ID NO 8
<211> LENGTH: 189
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 8

Met Ala Ser Pro Phe Ala Leu Leu Met Val Leu Val Val Leu Ser Cys
1 5 10 15

Lys Ser Ser Cys Ser Leu Gly Cys Asp Leu Pro Glu Thr His Ser Leu
20 25 30

Asp Asn Arg Arg Thr Leu Met Leu Leu Ala Gln Met Ser Arg Ile Ser
35 40 45

Pro Ser Ser Cys Leu Met Asp Arg His Asp Phe Gly Phe Pro Gln Glu
50 55 60

Glu Phe Asp Gly Asn Gln Phe Gln Lys Ala Pro Ala Ile Ser Val Leu
65 70 75 80

His Glu Leu Ile Gln Gln Ile Phe Asn Leu Phe Thr Thr Lys Asp Ser
85 90 95

Ser Ala Ala Trp Asp Glu Asp Leu Leu Asp Lys Phe Cys Thr Glu Leu
100 105 110

Tyr Gln Gln Leu Asn Asp Leu Glu Ala Cys Val Met Gln Glu Glu Arg
115 120 125

Val Gly Glu Thr Pro Leu Met Asn Val Asp Ser Ile Leu Ala Val Lys
130 135 140

Lys Tyr Phe Arg Arg Ile Thr Leu Tyr Leu Thr Glu Lys Lys Tyr Ser
145 150 155 160

Pro Cys Ala Trp Glu Val Val Arg Ala Glu Ile Met Arg Ser Leu Ser
165 170 175

Leu Ser Thr Asn Leu Gln Glu Arg Leu Arg Arg Lys Glu
180 185

<210> SEQ ID NO 9
<211> LENGTH: 876
<212> TYPE: DNA
<213> ORGANISM: Homo sapiens
<220> FEATURE:
<221> NAME/KEY: CDS
<222> LOCATION: (1)...(510)

<400> SEQUENCE: 9

ctg cct ctg ggc tgt gat ctg cct cag gcc cac agc gtg ggt aac agg 48
Leu Pro Leu Gly Cys Asp Leu Pro Gln Ala His Ser Val Gly Asn Arg
1 5 10 15

agg gcc ttc ata ctc ctg aca caa atg agg aga atc tct cct ttt tct 96
Arg Ala Phe Ile Leu Leu Thr Gln Met Arg Arg Ile Ser Pro Phe Ser
20 25 30

tac ctg aag gac aga cat gac ttt gat ttt cca tca tca ggt gtt tca 144
Tyr Leu Lys Asp Arg His Asp Phe Asp Phe Pro Ser Ser Gly Val Ser
35 40 45

tgg caa cca ctt cca gaa ggt tca agc tat ctt cct ttt cca tga gat 192
Trp Gln Pro Leu Pro Glu Gly Ser Ser Tyr Leu Pro Phe Pro * Asp
50 55 60

gat gca gca gac ctt caa cct ctt cag cac aaa gga ctc atc tga tac 240
Asp Ala Ala Asp Leu Gln Pro Leu Gln His Lys Gly Leu Ile * Tyr
65 70 75

ttg gga tga gac cct ttt aga caa atc cta cac tga act tta cca gca 288

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```
Leu Gly  *  Asp Pro Phe Arg Gln Ile Leu His  *  Thr Leu Pro Ala
     80                      85                      90

gct gaa tga cct gga agc ctg tgt gat gta gaa ggt tgg agt gga aga      336
Ala Glu  *  Pro Gly Ser Leu Cys Asp Val Glu Gly Trp Ser Gly Arg
            95                  100                 105

gac tcc cct gag gaa tgt gga ctc cat cct ggc tgt gag aaa ata ctt      384
Asp Ser Pro Glu Glu Cys Gly Leu His Pro Gly Cys Glu Lys Ile Leu
        110                 115                 120

tca aag aat cac tct tta tct gac aaa gaa gaa gta tag ccc ttg ttc      432
Ser Lys Asn His Ser Leu Ser Asp Lys Glu Glu Val  *  Pro Leu Phe
    125                 130                 135

ctg gga ggc tgt cag agc aga aat cat gag atc ctt ctc ttt atg aac      480
Leu Gly Gly Cys Gln Ser Arg Asn His Glu Ile Leu Leu Phe Met Asn
    140                 145                 150

gaa ctt gca gga aag att aag gag gaa gga atgaaaactg gttcaacatg        530
Glu Leu Ala Gly Lys Ile Lys Glu Glu Gly
155                 160

gaaatgagaa acatttccat gattaataca tcatctcaca cattcatgaa ttctgccatt    590

tgtcattttt gctatatcca tgacatgagt tgaatcaaaa ttttaaaatg ttttcaggaa    650

tgttaagcag catcatgttc agctgtacag gcactagttc cttacggatg atcatgctga    710

tggatctgtt tatctatttg tctaaataat tatttaacta tttataatat ttaaaatctt    770

cttttcatgt atcatgtatt tttactttgt ggttaatata acaacacatg ttctttatat    830

ttagtcaata tattactttg ctttttttcat taaattttta ctatgg                  876


<210> SEQ ID NO 10
<211> LENGTH: 62
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 10

Leu Pro Leu Gly Cys Asp Leu Pro Gln Ala His Ser Val Gly Asn Arg
 1               5                  10                  15

Arg Ala Phe Ile Leu Leu Thr Gln Met Arg Arg Ile Ser Pro Phe Ser
            20                  25                  30

Tyr Leu Lys Asp Arg His Asp Phe Asp Phe Pro Ser Ser Gly Val Ser
        35                  40                  45

Trp Gln Pro Leu Pro Glu Gly Ser Ser Tyr Leu Pro Phe Pro
    50                  55                  60


<210> SEQ ID NO 11
<211> LENGTH: 985
<212> TYPE: DNA
<213> ORGANISM: Homo sapiens
<220> FEATURE:
<221> NAME/KEY: CDS
<222> LOCATION: (9)...(575)

<400> SEQUENCE: 11

acatccca atg gcc ctg tcc ttt tct tta ctg atg gcc gtg ctg gtg ctc      50
         Met Ala Leu Ser Phe Ser Leu Leu Met Ala Val Leu Val Leu
          1               5                  10

agc tac aaa tcc atc tgt tct ctg ggc tgt gat ctg cct cag acc cac       98
Ser Tyr Lys Ser Ile Cys Ser Leu Gly Cys Asp Leu Pro Gln Thr His
 15                  20                  25                  30

agc ctg ggt aat agg agg gcc ttg ata ctc ctg gca caa atg gga aga      146
Ser Leu Gly Asn Arg Arg Ala Leu Ile Leu Leu Ala Gln Met Gly Arg
                 35                  40                  45

atc tct cct ttc tcc tgc ctg aag gac aga cat gac ttt gga ttc ccc      194
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```
Ile Ser Pro Phe Ser Cys Leu Lys Asp Arg His Asp Phe Gly Phe Pro
            50                  55                  60

caa gag gag ttt gat ggc aac cag ttc cag aag gct caa gcc atc tct      242
Gln Glu Glu Phe Asp Gly Asn Gln Phe Gln Lys Ala Gln Ala Ile Ser
        65                  70                  75

gtc ctc cat gag atg atc cag cag acc ttc aat ctc ttc agc aca aag      290
Val Leu His Glu Met Ile Gln Gln Thr Phe Asn Leu Phe Ser Thr Lys
    80                  85                  90

gac tca tct gct act tgg gaa cag agc ctc cta gaa aaa ttt tcc act      338
Asp Ser Ser Ala Thr Trp Glu Gln Ser Leu Leu Glu Lys Phe Ser Thr
 95                 100                 105                 110

gaa ctt aac cag cag ctg aat gac atg gaa gcc tgc gtg ata cag gag      386
Glu Leu Asn Gln Gln Leu Asn Asp Met Glu Ala Cys Val Ile Gln Glu
                115                 120                 125

gtt ggg gtg gaa gag act ccc ctg atg aat gtg gac tcc atc ttg gct      434
Val Gly Val Glu Glu Thr Pro Leu Met Asn Val Asp Ser Ile Leu Ala
            130                 135                 140

gtg aag aaa tac ttc caa aga atc act ctt tat ctg aca gag aag aaa      482
Val Lys Lys Tyr Phe Gln Arg Ile Thr Leu Tyr Leu Thr Glu Lys Lys
        145                 150                 155

tac agc cct tgt gct tgg gag gtt gtc aga gca gaa atc atg aga tcc      530
Tyr Ser Pro Cys Ala Trp Glu Val Val Arg Ala Glu Ile Met Arg Ser
    160                 165                 170

ttc tct tta tca aaa att ttt caa gaa aga tta agg agg aag gaa          575
Phe Ser Leu Ser Lys Ile Phe Gln Glu Arg Leu Arg Arg Lys Glu
175                 180                 185

tgaaaccgtt tcaacatgga aatgatctgt attgactaat acaccagtcc acacttctat    635

gacttctgcc atttcaaaga ctcatttctc ctataaccac cgcatgagtt gaatcaaaat    695

tttcagatct tttcaggagt gtaaggaaac atcatgttta cctgtgcagg cactagtcct    755

ttacagatga ccatgctgat agatctaatt atctatctat tgaaatattt atttatttat    815

tagatttaaa ttatttttgt ccatgtaata ttatgtgtac ttttacattg tgttatatca    875

aaatatgtta atctaatatt tagtcaatat attattttct ttttattaat ttttactatt    935

aaaacttctt atattatttg gttattcttt aataaagaaa ttccaagccc               985


<210> SEQ ID NO 12
<211> LENGTH: 189
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 12

Met Ala Leu Ser Phe Ser Leu Leu Met Ala Val Leu Val Leu Ser Tyr
 1               5                  10                  15

Lys Ser Ile Cys Ser Leu Gly Cys Asp Leu Pro Gln Thr His Ser Leu
            20                  25                  30

Gly Asn Arg Arg Ala Leu Ile Leu Leu Ala Gln Met Gly Arg Ile Ser
        35                  40                  45

Pro Phe Ser Cys Leu Lys Asp Arg His Asp Phe Gly Phe Pro Gln Glu
    50                  55                  60

Glu Phe Asp Gly Asn Gln Phe Gln Lys Ala Gln Ala Ile Ser Val Leu
65                  70                  75                  80

His Glu Met Ile Gln Gln Thr Phe Asn Leu Phe Ser Thr Lys Asp Ser
                85                  90                  95

Ser Ala Thr Trp Glu Gln Ser Leu Leu Glu Lys Phe Ser Thr Glu Leu
            100                 105                 110

Asn Gln Gln Leu Asn Asp Met Glu Ala Cys Val Ile Gln Glu Val Gly
```

-continued

```
        115                 120                 125

Val Glu Glu Thr Pro Leu Met Asn Val Asp Ser Ile Leu Ala Val Lys
    130                 135                 140

Lys Tyr Phe Gln Arg Ile Thr Leu Tyr Leu Thr Glu Lys Lys Tyr Ser
145                 150                 155                 160

Pro Cys Ala Trp Glu Val Val Arg Ala Glu Ile Met Arg Ser Phe Ser
                165                 170                 175

Leu Ser Lys Ile Phe Gln Glu Arg Leu Arg Arg Lys Glu
            180                 185
```

<210> SEQ ID NO 13
<211> LENGTH: 760
<212> TYPE: DNA
<213> ORGANISM: Homo sapiens
<220> FEATURE:
<221> NAME/KEY: CDS
<222> LOCATION: (1)...(399)

<400> SEQUENCE: 13

```
cat gac ttt gga ttt cct cag gag gag ttt gat ggc aac cag ttc cag       48
His Asp Phe Gly Phe Pro Gln Glu Glu Phe Asp Gly Asn Gln Phe Gln
 1               5                  10                  15

aag gct caa gcc atc tct gtc ctc cat gag atg atc cag cag acc ttc       96
Lys Ala Gln Ala Ile Ser Val Leu His Glu Met Ile Gln Gln Thr Phe
            20                  25                  30

aat ctc ttc agc aca aag gac tca tct gct act tgg gat gag aca ctt      144
Asn Leu Phe Ser Thr Lys Asp Ser Ser Ala Thr Trp Asp Glu Thr Leu
        35                  40                  45

cta gac aaa ttc tac act gaa ctt tac cag cag ctg aat gac ctg gaa      192
Leu Asp Lys Phe Tyr Thr Glu Leu Tyr Gln Gln Leu Asn Asp Leu Glu
    50                  55                  60

gcc tgt atg atg cag gag gtt gga gtg gaa gac act cct ctg atg aat      240
Ala Cys Met Met Gln Glu Val Gly Val Glu Asp Thr Pro Leu Met Asn
65                  70                  75                  80

gtg gac tct atc ctg act gtg aga aaa tac ttt caa aga atc acc ctc      288
Val Asp Ser Ile Leu Thr Val Arg Lys Tyr Phe Gln Arg Ile Thr Leu
                85                  90                  95

tat ctg aca gag aag aaa tac agc cct tgt gca tgg gag gtt gtc aga      336
Tyr Leu Thr Glu Lys Lys Tyr Ser Pro Cys Ala Trp Glu Val Val Arg
            100                 105                 110

gca gaa atc atg aga tcc ttc tct tta tca gca aac ttg caa gaa aga      384
Ala Glu Ile Met Arg Ser Phe Ser Leu Ser Ala Asn Leu Gln Glu Arg
        115                 120                 125

tta agg agg aag gaa tgaaaactgg ttcaacatcg aaatgattct cattgactag      439
Leu Arg Arg Lys Glu
    130

tacaccattt cacacttctt gagttctgcc gtttcaaata ttaatttctg ctatatccat    499

gacttgagtt gaatcaaaat tttcaaacgt tttcacacgt gttaagcaac acttctttag    559

ctgcacaggg actagtcttt tacagatgat catgctgaca tctattcttc tatttatcgt    619

catcattgtc gttttactac tattaatatt tatattatta ttgtttcatg ttatttttat    679

gtttagtttt agtttgtggt taatataaca aaatatgttt tgtggtcata tattaatttg    739

ctttttatta aattagtttg t                                              760
```

<210> SEQ ID NO 14
<211> LENGTH: 133
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens

-continued

<400> SEQUENCE: 14

```
His Asp Phe Gly Phe Pro Gln Glu Glu Phe Asp Gly Asn Gln Phe Gln
 1               5                  10                  15

Lys Ala Gln Ala Ile Ser Val Leu His Glu Met Ile Gln Gln Thr Phe
            20                  25                  30

Asn Leu Phe Ser Thr Lys Asp Ser Ser Ala Thr Trp Asp Glu Thr Leu
        35                  40                  45

Leu Asp Lys Phe Tyr Thr Glu Leu Tyr Gln Gln Leu Asn Asp Leu Glu
    50                  55                  60

Ala Cys Met Met Gln Glu Val Gly Val Glu Asp Thr Pro Leu Met Asn
65                  70                  75                  80

Val Asp Ser Ile Leu Thr Val Arg Lys Tyr Phe Gln Arg Ile Thr Leu
                85                  90                  95

Tyr Leu Thr Glu Lys Lys Tyr Ser Pro Cys Ala Trp Glu Val Val Arg
            100                 105                 110

Ala Glu Ile Met Arg Ser Phe Ser Leu Ser Ala Asn Leu Gln Glu Arg
        115                 120                 125

Leu Arg Arg Lys Glu
    130
```

<210> SEQ ID NO 15
<211> LENGTH: 985
<212> TYPE: DNA
<213> ORGANISM: Homo sapiens
<220> FEATURE:
<221> NAME/KEY: CDS
<222> LOCATION: (57)...(623)

<400> SEQUENCE: 15

```
ccaaggttca gtgttacccc tcatcaacca gcccagcagc atcttcggga ttccca atg     59
                                                              Met
                                                               1

gca ttg ccc ttt gct tta atg atg gcc cta gtg gtg ctc agc tgc aag      107
Ala Leu Pro Phe Ala Leu Met Met Ala Leu Val Val Leu Ser Cys Lys
            5                  10                  15

tca agc tgc tct ctg ggc tgt aat ctg tct caa acc cac agc ctg aat      155
Ser Ser Cys Ser Leu Gly Cys Asn Leu Ser Gln Thr His Ser Leu Asn
        20                  25                  30

aac agg agg act ttg atg ctc atg gca caa atg agg aga atc tct cct      203
Asn Arg Arg Thr Leu Met Leu Met Ala Gln Met Arg Arg Ile Ser Pro
    35                  40                  45

ttc tcc tgc ctg aag gac aga cat gac ttt gaa ttt ccc cag gag gaa      251
Phe Ser Cys Leu Lys Asp Arg His Asp Phe Glu Phe Pro Gln Glu Glu
50                  55                  60                  65

ttt gat ggc aac cag ttc cag aaa gct caa gcc atc tct gtc ctc cat      299
Phe Asp Gly Asn Gln Phe Gln Lys Ala Gln Ala Ile Ser Val Leu His
                70                  75                  80

gag atg atg cag cag acc ttc aat ctc ttc agc aca aag aac tca tct      347
Glu Met Met Gln Gln Thr Phe Asn Leu Phe Ser Thr Lys Asn Ser Ser
            85                  90                  95

gct gct tgg gat gag acc ctc cta gaa aaa ttc tac att gaa ctt ttc      395
Ala Ala Trp Asp Glu Thr Leu Leu Glu Lys Phe Tyr Ile Glu Leu Phe
        100                 105                 110

cag caa atg aat gac ctg gaa gcc tgt gtg ata cag gag gtt ggg gtg      443
Gln Gln Met Asn Asp Leu Glu Ala Cys Val Ile Gln Glu Val Gly Val
    115                 120                 125

gaa gag act ccc ctg atg aat gag gac tcc atc ctg gct gtg aag aaa      491
Glu Glu Thr Pro Leu Met Asn Glu Asp Ser Ile Leu Ala Val Lys Lys
130                 135                 140                 145
```

-continued

```
tac ttc caa aga atc act ctt tat ctg atg gag aag aaa tac agc cct      539
Tyr Phe Gln Arg Ile Thr Leu Tyr Leu Met Glu Lys Lys Tyr Ser Pro
                150                 155                 160

tgt gcc tgg gag gtt gtc aga gca gaa atc atg aga tcc ttc tct ttt      587
Cys Ala Trp Glu Val Val Arg Ala Glu Ile Met Arg Ser Phe Ser Phe
            165                 170                 175

tca aca aac ttg caa aaa aga tta agg agg aag gat tgaaaactgg           633
Ser Thr Asn Leu Gln Lys Arg Leu Arg Arg Lys Asp
        180                 185

ttcatcatgg aaatgattct cattgactaa tacatcatct cacactttca tgttcttcca    693

tttcaaagac tcacttctat aaccaccaca agttgaatca aaatttccaa atgttttcag    753

gagtgttaag aagcatcgtg tttacctgtg caggcactag tcctttacag atgaccattc    813

tgatgtctcc tttcatctat ttatttaaat atttatttat ttaactattt ttattattta    873

aattattttt tatgtaatat catgagtacc tttacattgt ggttaatgta acaaatatgt    933

tcttcatatt tagccaatat attaatttcc tttttcatta aatttttact at           985
```

<210> SEQ ID NO 16
<211> LENGTH: 189
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 16

```
Met Ala Leu Pro Phe Ala Leu Met Met Ala Leu Val Val Leu Ser Cys
 1               5                  10                  15

Lys Ser Ser Cys Ser Leu Gly Cys Asn Leu Ser Gln Thr His Ser Leu
            20                  25                  30

Asn Asn Arg Arg Thr Leu Met Leu Met Ala Gln Met Arg Arg Ile Ser
        35                  40                  45

Pro Phe Ser Cys Leu Lys Asp Arg His Asp Phe Glu Phe Pro Gln Glu
    50                  55                  60

Glu Phe Asp Gly Asn Gln Phe Gln Lys Ala Gln Ala Ile Ser Val Leu
65                  70                  75                  80

His Glu Met Met Gln Gln Thr Phe Asn Leu Phe Ser Thr Lys Asn Ser
                85                  90                  95

Ser Ala Ala Trp Asp Glu Thr Leu Leu Glu Lys Phe Tyr Ile Glu Leu
            100                 105                 110

Phe Gln Gln Met Asn Asp Leu Glu Ala Cys Val Ile Gln Glu Val Gly
        115                 120                 125

Val Glu Glu Thr Pro Leu Met Asn Glu Asp Ser Ile Leu Ala Val Lys
    130                 135                 140

Lys Tyr Phe Gln Arg Ile Thr Leu Tyr Leu Met Glu Lys Lys Tyr Ser
145                 150                 155                 160

Pro Cys Ala Trp Glu Val Val Arg Ala Glu Ile Met Arg Ser Phe Ser
                165                 170                 175

Phe Ser Thr Asn Leu Gln Lys Arg Leu Arg Arg Lys Asp
            180                 185
```

<210> SEQ ID NO 17
<211> LENGTH: 188
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 17

```
Met Ala Leu Thr Phe Ala Leu Leu Val Ala Leu Leu Val Leu Ser Cys
 1               5                  10                  15
```

-continued

```
Lys Ser Ser Cys Ser Val Gly Cys Asp Leu Pro Gln Thr His Ser Leu
            20                  25                  30

Gly Ser Arg Arg Thr Leu Met Leu Leu Ala Gln Met Arg Lys Ile Ser
        35                  40                  45

Leu Phe Ser Cys Leu Lys Asp Arg His Asp Phe Gly Phe Pro Gln Glu
    50                  55                  60

Glu Phe Gly Asn Gln Phe Gln Lys Ala Glu Thr Ile Pro Val Leu His
65                  70                  75                  80

Glu Met Ile Gln Gln Ile Phe Asn Leu Phe Ser Thr Lys Asp Ser Ser
                85                  90                  95

Ala Ala Trp Asp Glu Thr Leu Leu Asp Lys Phe Tyr Thr Glu Leu Tyr
            100                 105                 110

Gln Gln Leu Asn Asp Leu Glu Ala Cys Val Ile Gln Gly Val Gly Val
        115                 120                 125

Thr Glu Thr Pro Leu Met Lys Glu Asp Ser Ile Leu Ala Val Arg Lys
    130                 135                 140

Tyr Phe Gln Arg Ile Thr Leu Tyr Leu Lys Glu Lys Lys Tyr Ser Pro
145                 150                 155                 160

Cys Ala Trp Glu Val Val Arg Ala Glu Ile Met Arg Ser Phe Ser Leu
                165                 170                 175

Ser Thr Asn Leu Gln Glu Ser Leu Arg Ser Lys Glu
            180                 185


<210> SEQ ID NO 18
<211> LENGTH: 189
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 18

Met Ala Leu Thr Phe Tyr Leu Met Val Ala Leu Val Val Leu Ser Tyr
 1               5                  10                  15

Lys Ser Phe Ser Ser Leu Gly Cys Asp Leu Pro Gln Thr His Ser Leu
            20                  25                  30

Gly Asn Arg Arg Ala Leu Ile Leu Leu Ala Gln Met Arg Arg Ile Ser
        35                  40                  45

Pro Phe Ser Cys Leu Lys Asp Arg His Asp Phe Glu Phe Pro Gln Glu
    50                  55                  60

Glu Phe Asp Asp Lys Gln Phe Gln Lys Ala Gln Ala Ile Ser Val Leu
65                  70                  75                  80

His Glu Met Ile Gln Gln Thr Phe Asn Leu Phe Ser Thr Lys Asp Ser
                85                  90                  95

Ser Ala Ala Leu Asp Glu Thr Leu Leu Asp Glu Phe Tyr Ile Glu Leu
            100                 105                 110

Asp Gln Gln Leu Asn Asp Leu Glu Val Leu Cys Asp Gln Glu Val Gly
        115                 120                 125

Val Ile Glu Ser Pro Leu Met Tyr Glu Asp Ser Ile Leu Ala Val Arg
    130                 135                 140

Lys Tyr Phe Gln Arg Ile Thr Leu Tyr Leu Thr Glu Lys Lys Tyr Ser
145                 150                 155                 160

Ser Cys Ala Trp Glu Val Val Arg Ala Glu Ile Met Arg Ser Phe Ser
                165                 170                 175

Leu Ser Ile Asn Leu Gln Lys Arg Leu Lys Ser Lys Glu
            180                 185
```

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<210> SEQ ID NO 19
<211> LENGTH: 189
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 19

Met Ala Leu Ser Phe Ser Leu Leu Met Ala Val Leu Val Leu Ser Tyr
1 5 10 15

Lys Ser Ile Cys Ser Leu Gly Cys Asp Leu Pro Gln Thr His Ser Leu
20 25 30

Gly Asn Arg Arg Ala Leu Ile Leu Leu Gly Gln Met Gly Arg Ile Ser
35 40 45

Pro Phe Ser Cys Leu Lys Asp Arg His Asp Phe Arg Ile Pro Gln Glu
50 55 60

Glu Phe Asp Gly Asn Gln Phe Gln Lys Ala Gln Ala Ile Ser Val Leu
65 70 75 80

His Glu Met Ile Gln Gln Thr Phe Asn Leu Phe Ser Thr Glu Asp Ser
85 90 95

Ser Ala Ala Trp Glu Gln Ser Leu Leu Glu Lys Phe Ser Thr Glu Leu
100 105 110

Tyr Gln Gln Leu Asn Asp Leu Glu Ala Cys Val Ile Gln Glu Val Gly
115 120 125

Val Glu Glu Thr Pro Leu Met Asn Glu Asp Ser Ile Leu Ala Val Arg
130 135 140

Lys Tyr Phe Gln Arg Ile Thr Leu Tyr Leu Ile Glu Arg Lys Tyr Ser
145 150 155 160

Pro Cys Ala Trp Glu Val Val Arg Ala Glu Ile Met Arg Ser Leu Ser
165 170 175

Phe Ser Thr Asn Leu Gln Lys Arg Leu Arg Arg Lys Asp
180 185

<210> SEQ ID NO 20
<211> LENGTH: 189
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 20

Met Ala Ser Pro Phe Ala Leu Leu Met Val Leu Val Val Leu Ser Cys
1 5 10 15

Lys Ser Ser Cys Ser Leu Gly Cys Asp Leu Pro Glu Thr His Ser Leu
20 25 30

Asp Asn Arg Arg Thr Leu Met Leu Leu Ala Gln Met Ser Arg Ile Ser
35 40 45

Pro Ser Ser Cys Leu Met Asp Arg His Asp Phe Gly Phe Pro Gln Glu
50 55 60

Glu Phe Asp Gly Asn Gln Phe Gln Lys Ala Pro Ala Ile Ser Val Leu
65 70 75 80

His Glu Leu Ile Gln Gln Ile Phe Asn Leu Phe Thr Thr Lys Asp Ser
85 90 95

Ser Ala Ala Trp Asp Glu Asp Leu Leu Asp Lys Phe Cys Thr Glu Leu
100 105 110

Tyr Gln Gln Leu Asn Asp Leu Glu Ala Cys Val Met Gln Glu Glu Arg
115 120 125

Val Gly Glu Thr Pro Leu Met Asn Val Asp Ser Ile Leu Ala Val Lys
130 135 140

Lys Tyr Phe Arg Arg Ile Thr Leu Tyr Leu Thr Glu Lys Lys Tyr Ser

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145                 150                 155                 160

Pro Cys Ala Trp Glu Val Val Arg Ala Glu Ile Met Arg Ser Leu Ser
                165                 170                 175

Leu Ser Thr Asn Leu Gln Glu Arg Leu Arg Arg Lys Glu
            180                 185


<210> SEQ ID NO 21
<211> LENGTH: 105
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 21

Leu Pro Leu Gly Cys Asp Leu Pro Gln Ala His Ser Val Gly Asn Arg
 1               5                  10                  15

Arg Ala Phe Ile Leu Leu Thr Gln Met Arg Arg Ile Ser Pro Phe Ser
            20                  25                  30

Tyr Leu Lys Asp Arg His Asp Phe Asp Phe Pro His Gln Val Phe His
        35                  40                  45

Gly Asn His Phe Gln Lys Val Gln Ala Ile Phe Leu Phe His Glu Met
    50                  55                  60

Met Gln Gln Thr Phe Asn Leu Phe Ser Thr Lys Asp Ser Ser Asp Thr
65                  70                  75                  80

Trp Asp Glu Thr Leu Leu Asp Lys Ser Tyr Thr Glu Leu Tyr Gln Gln
                85                  90                  95

Leu Asn Asp Leu Glu Ala Cys Val Met
            100                 105


<210> SEQ ID NO 22
<211> LENGTH: 189
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 22

Met Ala Leu Ser Phe Ser Leu Leu Met Ala Val Leu Val Leu Ser Tyr
 1               5                  10                  15

Lys Ser Ile Cys Ser Leu Gly Cys Asp Leu Pro Gln Thr His Ser Leu
            20                  25                  30

Gly Asn Arg Arg Ala Leu Ile Leu Leu Ala Gln Met Gly Arg Ile Ser
        35                  40                  45

Pro Phe Ser Cys Leu Lys Asp Arg His Asp Phe Gly Phe Pro Gln Glu
    50                  55                  60

Glu Phe Asp Gly Asn Gln Phe Gln Lys Ala Gln Ala Ile Ser Val Leu
65                  70                  75                  80

His Glu Met Ile Gln Gln Thr Phe Asn Leu Phe Ser Thr Lys Asp Ser
                85                  90                  95

Ser Ala Thr Trp Glu Gln Ser Leu Leu Glu Lys Phe Ser Thr Glu Leu
            100                 105                 110

Asn Gln Gln Leu Asn Asp Met Glu Ala Cys Val Ile Gln Glu Val Gly
        115                 120                 125

Val Glu Glu Thr Pro Leu Met Asn Val Asp Ser Ile Leu Ala Val Lys
    130                 135                 140

Lys Tyr Phe Gln Arg Ile Thr Leu Tyr Leu Thr Glu Lys Lys Tyr Ser
145                 150                 155                 160

Pro Cys Ala Trp Glu Val Val Arg Ala Glu Ile Met Arg Ser Phe Ser
                165                 170                 175

Leu Ser Lys Ile Phe Gln Glu Arg Leu Arg Arg Lys Glu
```

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180 185

<210> SEQ ID NO 23
<211> LENGTH: 133
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 23

His Asp Phe Gly Phe Pro Gln Glu Glu Phe Asp Gly Asn Gln Phe Gln
1 5 10 15

Lys Ala Gln Ala Ile Ser Val Leu His Glu Met Ile Gln Gln Thr Phe
20 25 30

Asn Leu Phe Ser Thr Lys Asp Ser Ser Ala Thr Trp Asp Glu Thr Leu
35 40 45

Leu Asp Lys Phe Tyr Thr Glu Leu Tyr Gln Gln Leu Asn Asp Leu Glu
50 55 60

Ala Cys Met Met Gln Glu Val Gly Val Glu Asp Thr Pro Leu Met Asn
65 70 75 80

Val Asp Ser Ile Leu Thr Val Arg Lys Tyr Phe Gln Arg Ile Thr Leu
85 90 95

Tyr Leu Thr Glu Lys Lys Tyr Ser Pro Cys Ala Trp Glu Val Val Arg
100 105 110

Ala Glu Ile Met Arg Ser Phe Ser Leu Ser Ala Asn Leu Gln Glu Arg
115 120 125

Leu Arg Arg Lys Glu
130

<210> SEQ ID NO 24
<211> LENGTH: 189
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 24

Met Ala Leu Pro Phe Ser Leu Met Met Ala Leu Val Val Leu Ser Cys
1 5 10 15

Lys Ser Ser Cys Ser Leu Gly Cys Asn Leu Ser Gln Thr His Ser Leu
20 25 30

Asn Asn Arg Arg Thr Leu Met Leu Met Ala Gln Met Arg Arg Ile Ser
35 40 45

Pro Phe Ser Cys Leu Lys Asp Arg His Asp Phe Glu Phe Pro Gln Glu
50 55 60

Glu Phe Asp Gly Asn Gln Phe Gln Lys Ala Gln Ala Ile Ser Val Leu
65 70 75 80

His Glu Met Met Gln Gln Thr Phe Asn Leu Phe Ser Thr Lys Asn Ser
85 90 95

Ser Ala Ala Trp Asp Glu Thr Leu Leu Glu Lys Phe Tyr Ile Glu Leu
100 105 110

Phe Gln Gln Met Asn Asp Leu Glu Ala Cys Val Ile Gln Glu Val Gly
115 120 125

Val Glu Glu Thr Pro Leu Met Asn Glu Asp Ser Ile Leu Ala Val Arg
130 135 140

Lys Tyr Phe Gln Arg Ile Thr Leu Tyr Leu Met Glu Lys Lys Tyr Ser
145 150 155 160

Pro Cys Ala Trp Glu Val Val Arg Ala Glu Ile Met Arg Ser Phe Ser
165 170 175

Phe Ser Thr Asn Leu Gln Lys Arg Leu Arg Arg Lys Asp

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180 185

<210> SEQ ID NO 25
<211> LENGTH: 1107
<212> TYPE: DNA
<213> ORGANISM: Homo sapiens
<220> FEATURE:
<221> NAME/KEY: CDS
<222> LOCATION: (111)...(674)

<400> SEQUENCE: 25

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gtatgttccc tatttaaggc taggcacaaa gcaaggtctt cagagaacct ggagcctaag      60

gtttaggctc acccatttca accagtctag cagcatctgc aacatctaca atg gcc        116
                                                          Met Ala
                                                           1

ttg acc ttt gct tta ctg gtg gcc ctc ctg gtg ctc agc tgc aag tca       164
Leu Thr Phe Ala Leu Leu Val Ala Leu Leu Val Leu Ser Cys Lys Ser
         5                  10                  15

agc tgc tct gtg ggc tgt gat ctg cct caa acc cac agc ctg ggt agc       212
Ser Cys Ser Val Gly Cys Asp Leu Pro Gln Thr His Ser Leu Gly Ser
     20                  25                  30

agg agg acc ttg atg ctc ctg gca cag atg agg aga atc tct ctt ttc       260
Arg Arg Thr Leu Met Leu Leu Ala Gln Met Arg Arg Ile Ser Leu Phe
 35                  40                  45                  50

tcc tgc ttg aag gac aga cat gac ttt gga ttt ccc cag gag gag ttt       308
Ser Cys Leu Lys Asp Arg His Asp Phe Gly Phe Pro Gln Glu Glu Phe
                 55                  60                  65

ggc aac cag ttc caa aag gct gaa acc atc cct gtc ctc cat gag atg       356
Gly Asn Gln Phe Gln Lys Ala Glu Thr Ile Pro Val Leu His Glu Met
             70                  75                  80

atc cag cag atc ttc aat ctc ttc agc aca aag gac tca tct gct gct       404
Ile Gln Gln Ile Phe Asn Leu Phe Ser Thr Lys Asp Ser Ser Ala Ala
         85                  90                  95

tgg gat gag acc ctc cta gac aaa ttc tac act gaa ctc tac cag cag       452
Trp Asp Glu Thr Leu Leu Asp Lys Phe Tyr Thr Glu Leu Tyr Gln Gln
    100                 105                 110

ctg aat gac ctg gaa gcc tgt gtg ata cag ggg gtg ggg gtg aca gag       500
Leu Asn Asp Leu Glu Ala Cys Val Ile Gln Gly Val Gly Val Thr Glu
115                 120                 125                 130

act ccc ctg atg aag gag gac tcc att ctg gct gtg agg aaa tac ttc       548
Thr Pro Leu Met Lys Glu Asp Ser Ile Leu Ala Val Arg Lys Tyr Phe
                135                 140                 145

caa aga atc act ctc tat ctg aaa gag aag aaa tac agc cct tgt gcc       596
Gln Arg Ile Thr Leu Tyr Leu Lys Glu Lys Lys Tyr Ser Pro Cys Ala
            150                 155                 160

tgg gag gtt gtc aga gca gaa atc atg aga tct ttt tct ttg tca aca       644
Trp Glu Val Val Arg Ala Glu Ile Met Arg Ser Phe Ser Leu Ser Thr
        165                 170                 175

aac ttg caa gaa agt tta aga agt aag gaa tgaaaactgg ttcaacatgg        694
Asn Leu Gln Glu Ser Leu Arg Ser Lys Glu
    180                 185

aaatgatttt cattgattcg tatgccagct caccttttta tgatctgcca tttcaaagac    754

tcatgtttct gctatgacca tgacacgatt taaatctttt caaatgtttt taggagtatt    814

aatcaacatt gtattcagct cttaaggcac tagtccctta cagaggacca tgctgactga    874

tccattatct atttaaatat ttttaaaata ttatttattt aactatttat aaaacaactt    934

atttttgttc atattatgtc atgtgcacct ttgcacagtg gttaatgtaa taaaatgtgt    994

tctttgtatt tggtaaattt attttgtgtt gttcattgaa cttttgctat ggaacttttg   1054
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tacttgttta ttctttaaaa tgaaattcca agcctaattg tgcaacctga tta 1107

<210> SEQ ID NO 26
<211> LENGTH: 188
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 26

Met Ala Leu Thr Phe Ala Leu Leu Val Ala Leu Leu Val Leu Ser Cys
1 5 10 15

Lys Ser Ser Cys Ser Val Gly Cys Asp Leu Pro Gln Thr His Ser Leu
20 25 30

Gly Ser Arg Arg Thr Leu Met Leu Leu Ala Gln Met Arg Arg Ile Ser
35 40 45

Leu Phe Ser Cys Leu Lys Asp Arg His Asp Phe Gly Phe Pro Gln Glu
50 55 60

Glu Phe Gly Asn Gln Phe Gln Lys Ala Glu Thr Ile Pro Val Leu His
65 70 75 80

Glu Met Ile Gln Gln Ile Phe Asn Leu Phe Ser Thr Lys Asp Ser Ser
85 90 95

Ala Ala Trp Asp Glu Thr Leu Leu Asp Lys Phe Tyr Thr Glu Leu Tyr
100 105 110

Gln Gln Leu Asn Asp Leu Glu Ala Cys Val Ile Gln Gly Val Gly Val
115 120 125

Thr Glu Thr Pro Leu Met Lys Glu Asp Ser Ile Leu Ala Val Arg Lys
130 135 140

Tyr Phe Gln Arg Ile Thr Leu Tyr Leu Lys Glu Lys Lys Tyr Ser Pro
145 150 155 160

Cys Ala Trp Glu Val Val Arg Ala Glu Ile Met Arg Ser Phe Ser Leu
165 170 175

Ser Thr Asn Leu Gln Glu Ser Leu Arg Ser Lys Glu
180 185

<210> SEQ ID NO 27
<211> LENGTH: 1069
<212> TYPE: DNA
<213> ORGANISM: Homo sapiens
<220> FEATURE:
<221> NAME/KEY: CDS
<222> LOCATION: (111)...(677)

<400> SEQUENCE: 27

gtatgttccc tatttaaggc taggcacaaa gcaaggtctt cagagaacct ggagcctaag 60

gtttaggctc acccatttca accagtctag cagcatctgc aacatctaca atg gca 116
Met Ala
1

ttg ccc ttt gct tta atg atg gcc ctg gtg gtg ctc agc tgc aag tca 164
Leu Pro Phe Ala Leu Met Met Ala Leu Val Val Leu Ser Cys Lys Ser
5 10 15

agc tgc tct ctg ggc tgt aat ctg tct caa acc cac agc ctg aat aac 212
Ser Cys Ser Leu Gly Cys Asn Leu Ser Gln Thr His Ser Leu Asn Asn
20 25 30

agg agg act ttg atg ctc atg gca caa atg agg aga atc tct cct ttc 260
Arg Arg Thr Leu Met Leu Met Ala Gln Met Arg Arg Ile Ser Pro Phe
35 40 45 50

tcc tgc ctg aag gac aga cat gac ttt gaa ttt ccc cag gag gaa ttt 308
Ser Cys Leu Lys Asp Arg His Asp Phe Glu Phe Pro Gln Glu Glu Phe
55 60 65

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gat ggc aac cag ttc cag aaa gct caa gcc atc tct gtc ctc cat gag      356
Asp Gly Asn Gln Phe Gln Lys Ala Gln Ala Ile Ser Val Leu His Glu
             70                  75                  80

atg atg cag cag acc ttc aat ctc ttc agc aca aag aac tca tct gct      404
Met Met Gln Gln Thr Phe Asn Leu Phe Ser Thr Lys Asn Ser Ser Ala
         85                  90                  95

gct tgg gat gag acc ctc cta gaa aaa ttc tac att gaa ctt ttc cag      452
Ala Trp Asp Glu Thr Leu Leu Glu Lys Phe Tyr Ile Glu Leu Phe Gln
    100                 105                 110

caa atg aat gac ctg gaa gcc tgt gtg ata cag gag gtt ggg gtg gaa      500
Gln Met Asn Asp Leu Glu Ala Cys Val Ile Gln Glu Val Gly Val Glu
115                 120                 125                 130

gag act ccc ctg atg aat gag gac tcc atc ctg gct gtg aag aaa tac      548
Glu Thr Pro Leu Met Asn Glu Asp Ser Ile Leu Ala Val Lys Lys Tyr
                135                 140                 145

ttc caa aga atc act ctt tat ctg atg gag aag aaa tac agc cct tgt      596
Phe Gln Arg Ile Thr Leu Tyr Leu Met Glu Lys Lys Tyr Ser Pro Cys
            150                 155                 160

gcc tgg gag gtt gtc aga gca gaa atc atg aga tcc ctc tct ttt tca      644
Ala Trp Glu Val Val Arg Ala Glu Ile Met Arg Ser Leu Ser Phe Ser
        165                 170                 175

aca aac ttg caa aaa aga tta agg agg aag gat tgaaaagtgg ttcatcatgg    697
Thr Asn Leu Gln Lys Arg Leu Arg Arg Lys Asp
    180                 185

aaatgattct cattgactaa tacatcatct cacactttca tgagttcttc catttcaaag    757

actcacttct cctataacca ccacaagttg aatcaaaatt ttcaaatgtt ttcaggagtg    817

taaagaagca tcatgtatac ctgtgcaggc actagtcctt tacagatgac catgctgatg    877

tctcctttca tctatttatt taaatattta tttatttaac tatttttatt atttaaatta    937

ttttttatgt taatatcatg tgtaccttta cattgtggtt aatataacaa atatgttctt    997

catatttagc caatatatta atttcctttt tcattaaatt tttactatac aaaatttctg   1057

tgtttggtat tt                                                       1069


<210> SEQ ID NO 28
<211> LENGTH: 189
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 28

Met Ala Leu Pro Phe Ala Leu Met Met Ala Leu Val Val Leu Ser Cys
 1               5                  10                  15

Lys Ser Ser Cys Ser Leu Gly Cys Asn Leu Ser Gln Thr His Ser Leu
            20                  25                  30

Asn Asn Arg Arg Thr Leu Met Leu Met Ala Gln Met Arg Arg Ile Ser
        35                  40                  45

Pro Phe Ser Cys Leu Lys Asp Arg His Asp Phe Glu Phe Pro Gln Glu
    50                  55                  60

Glu Phe Asp Gly Asn Gln Phe Gln Lys Ala Gln Ala Ile Ser Val Leu
65                  70                  75                  80

His Glu Met Met Gln Gln Thr Phe Asn Leu Phe Ser Thr Lys Asn Ser
                85                  90                  95

Ser Ala Ala Trp Asp Glu Thr Leu Leu Glu Lys Phe Tyr Ile Glu Leu
            100                 105                 110

Phe Gln Gln Met Asn Asp Leu Glu Ala Cys Val Ile Gln Glu Val Gly
        115                 120                 125

Val Glu Glu Thr Pro Leu Met Asn Glu Asp Ser Ile Leu Ala Val Lys
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    130                 135                 140

Lys Tyr Phe Gln Arg Ile Thr Leu Tyr Leu Met Glu Lys Lys Tyr Ser
145                 150                 155                 160

Pro Cys Ala Trp Glu Val Val Arg Ala Glu Ile Met Arg Ser Leu Ser
                165                 170                 175

Phe Ser Thr Asn Leu Gln Lys Arg Leu Arg Arg Lys Asp
            180                 185
```

<210> SEQ ID NO 29
<211> LENGTH: 1112
<212> TYPE: DNA
<213> ORGANISM: Homo sapiens
<220> FEATURE:
<221> NAME/KEY: CDS
<222> LOCATION: (113)...(679)

<400> SEQUENCE: 29

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gtatgttcct tatttaagac ctatgcacag agcaaggtct tcagaaaacc tacaacccaa     60

ggttcagtgt tacccctcat caaccagccc agcagcatct tcagggttcc ca atg gcc    118
                                                          Met Ala
                                                           1

ctg tcc ttt tct tta ctg atg gcc gtg ctg gtg ctc agc tac aaa tcc      166
Leu Ser Phe Ser Leu Leu Met Ala Val Leu Val Leu Ser Tyr Lys Ser
         5                  10                  15

atc tgt tct cta ggc tgt gat ctg cct cag acc cac agc ctg ggt aat      214
Ile Cys Ser Leu Gly Cys Asp Leu Pro Gln Thr His Ser Leu Gly Asn
     20                  25                  30

agg agg gcc ttg ata ctc ctg gca caa atg gga aga atc tct cct ttc      262
Arg Arg Ala Leu Ile Leu Leu Ala Gln Met Gly Arg Ile Ser Pro Phe
 35                  40                  45                  50

tcc tgc ctg aag gac aga cct gac ttt gga ctt ccc cag gag gag ttt      310
Ser Cys Leu Lys Asp Arg Pro Asp Phe Gly Leu Pro Gln Glu Glu Phe
                 55                  60                  65

gat ggc aac cag ttc cag aag act caa gcc atc tct gtc ctc cat gag      358
Asp Gly Asn Gln Phe Gln Lys Thr Gln Ala Ile Ser Val Leu His Glu
             70                  75                  80

atg atc cag cag acc ttc aat ctc ttc agc aca gag gac tca tct gct      406
Met Ile Gln Gln Thr Phe Asn Leu Phe Ser Thr Glu Asp Ser Ser Ala
         85                  90                  95

gct tgg gaa cag agc ctc cta gaa aaa ttt tcc act gaa ctt tac cag      454
Ala Trp Glu Gln Ser Leu Leu Glu Lys Phe Ser Thr Glu Leu Tyr Gln
    100                 105                 110

caa ctg aat aac ctg gaa gca tgt gtg ata cag gag gtt ggg atg gaa      502
Gln Leu Asn Asn Leu Glu Ala Cys Val Ile Gln Glu Val Gly Met Glu
115                 120                 125                 130

gag act ccc ctg atg aat gag gac tcc atc ctg gct gtg agg aaa tac      550
Glu Thr Pro Leu Met Asn Glu Asp Ser Ile Leu Ala Val Arg Lys Tyr
                135                 140                 145

ttc caa aga atc act ctt tat cta aca gag aag aaa tac agc cct tca      598
Phe Gln Arg Ile Thr Leu Tyr Leu Thr Glu Lys Lys Tyr Ser Pro Ser
            150                 155                 160

gcc tgg gag gtt gtc aga gca gaa atc atg aga tct ctc tct ttt tca      646
Ala Trp Glu Val Val Arg Ala Glu Ile Met Arg Ser Leu Ser Phe Ser
        165                 170                 175

aca aac ttg caa aaa ata tta agg agg aag gat tgaaaactgg ttcaacatgg    699
Thr Asn Leu Gln Lys Ile Leu Arg Arg Lys Asp
    180                 185

caatgatcct gattgactaa tacattatct cacactttca tgagttcctc catttcaaag    759

actcacttct ataaccacca cgagttgaat caaaattttc aaatgttttc agcagtgtaa    819
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agaagcgtcg tgtatacctg tgcaggcact agtactttac agatgaccat gctgatgtct    879

ctgttcatct atttatttaa atatttattt aattattttt aagatttaaa ttatttttt    939

atgtaatatc atgtgtacct ttacattgtg gtgaatgtaa caatatatgt tcttcatatt    999

tagccaatat attaatttcc tttttcatta aatttttact atacaaaatt tcttgagttt   1059

gtttattctt aagaataaaa tgtcgaggct gactttacaa cctgacttaa aaa          1112
```

<210> SEQ ID NO 30
<211> LENGTH: 189
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 30

```
Met Ala Leu Ser Phe Ser Leu Leu Met Ala Val Leu Val Leu Ser Tyr
 1               5                  10                  15

Lys Ser Ile Cys Ser Leu Gly Cys Asp Leu Pro Gln Thr His Ser Leu
            20                  25                  30

Gly Asn Arg Arg Ala Leu Ile Leu Leu Ala Gln Met Gly Arg Ile Ser
        35                  40                  45

Pro Phe Ser Cys Leu Lys Asp Arg Pro Asp Phe Gly Leu Pro Gln Glu
    50                  55                  60

Glu Phe Asp Gly Asn Gln Phe Gln Lys Thr Gln Ala Ile Ser Val Leu
65                  70                  75                  80

His Glu Met Ile Gln Gln Thr Phe Asn Leu Phe Ser Thr Glu Asp Ser
                85                  90                  95

Ser Ala Ala Trp Glu Gln Ser Leu Leu Glu Lys Phe Ser Thr Glu Leu
            100                 105                 110

Tyr Gln Gln Leu Asn Asn Leu Glu Ala Cys Val Ile Gln Glu Val Gly
        115                 120                 125

Met Glu Glu Thr Pro Leu Met Asn Glu Asp Ser Ile Leu Ala Val Arg
    130                 135                 140

Lys Tyr Phe Gln Arg Ile Thr Leu Tyr Leu Thr Glu Lys Lys Tyr Ser
145                 150                 155                 160

Pro Ser Ala Trp Glu Val Val Arg Ala Glu Ile Met Arg Ser Leu Ser
                165                 170                 175

Phe Ser Thr Asn Leu Gln Lys Ile Leu Arg Arg Lys Asp
            180                 185
```

<210> SEQ ID NO 31
<211> LENGTH: 1109
<212> TYPE: DNA
<213> ORGANISM: Homo sapiens
<220> FEATURE:
<221> NAME/KEY: CDS
<222> LOCATION: (111)...(677)

<400> SEQUENCE: 31

```
gtatgttcac tatttaaggc ctatgcacag agcaaagtct tcagaaaacc tagaggccaa     60

agttcaaggt tacccatctc aagtagccta gcaacatttg caacatccca atg gcc       116
                                                          Met Ala
                                                           1

cgg tcc ttt tct tta ctg atg gtc gtg ctg gta ctc agc tac aaa tcc      164
Arg Ser Phe Ser Leu Leu Met Val Val Leu Val Leu Ser Tyr Lys Ser
 5                  10                  15

atc tgc tct ctg ggc tgt gat ctg cct cag acc cac agc ctg cgt aat      212
Ile Cys Ser Leu Gly Cys Asp Leu Pro Gln Thr His Ser Leu Arg Asn
     20                  25                  30
```

-continued

```
agg agg gcc ttg ata ctc ctg gca caa atg gga aga atc tct cct ttc      260
Arg Arg Ala Leu Ile Leu Leu Ala Gln Met Gly Arg Ile Ser Pro Phe
 35                  40                  45                  50

tcc tgc ttg aag gac aga cat gaa ttc aga ttc cca gag gag gag ttt      308
Ser Cys Leu Lys Asp Arg His Glu Phe Arg Phe Pro Glu Glu Glu Phe
                 55                  60                  65

gat ggc cac cag ttc cag aag act caa gcc atc tct gtc ctc cat gag      356
Asp Gly His Gln Phe Gln Lys Thr Gln Ala Ile Ser Val Leu His Glu
             70                  75                  80

atg atc cag cag acc ttc aat ctc ttc agc aca gag gac tca tct gct      404
Met Ile Gln Gln Thr Phe Asn Leu Phe Ser Thr Glu Asp Ser Ser Ala
         85                  90                  95

gct tgg gaa cag agc ctc cta gaa aaa ttt tcc act gaa ctt tac cag      452
Ala Trp Glu Gln Ser Leu Leu Glu Lys Phe Ser Thr Glu Leu Tyr Gln
    100                 105                 110

caa ctg aat gac ctg gaa gca tgt gtg ata cag gag gtt ggg gtg gaa      500
Gln Leu Asn Asp Leu Glu Ala Cys Val Ile Gln Glu Val Gly Val Glu
115                 120                 125                 130

gag act ccc ctg atg aat gag gac ttc atc ctg gct gtg agg aaa tac      548
Glu Thr Pro Leu Met Asn Glu Asp Phe Ile Leu Ala Val Arg Lys Tyr
                135                 140                 145

ttc caa aga atc act ctt tat cta atg gag aag aaa tac agc cct tgt      596
Phe Gln Arg Ile Thr Leu Tyr Leu Met Glu Lys Lys Tyr Ser Pro Cys
            150                 155                 160

gcc tgg gag gtt gtc aga gca gaa atc atg aga tcc ttc tct ttt tca      644
Ala Trp Glu Val Val Arg Ala Glu Ile Met Arg Ser Phe Ser Phe Ser
        165                 170                 175

aca aac ttg aaa aaa gga tta agg agg aag gat tgaaaactgg ttcatcatgg    697
Thr Asn Leu Lys Lys Gly Leu Arg Arg Lys Asp
    180                 185

aaatgattct cattgactaa tgcatcatct cacactttca tgagttcttc catttcaaag    757

actcacttct ataaccacca caagttgaat caaaatttcc aaatgttttc aggagtgtta    817

agaagcatcg tgtttacctg tgcaggcact agtcctttac agatgaccat tctgatgtct    877

cctttcatct atttatttaa atatttattt atttaactat ttttattatt taaattattt    937

tttatgtaat atcatatgta cctttacatt gtggttaatg taacaaatat gttcttcata    997

tttagccaat atattaattt ccttttttcat taaattttta ctatacaaaa tttcttgtgt   1057

ttgtttattt tttaagatta aatgccaagc ctgactgtat aacctgactt aa           1109


<210> SEQ ID NO 32
<211> LENGTH: 189
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 32

Met Ala Arg Ser Phe Ser Leu Leu Met Val Val Leu Val Leu Ser Tyr
 1               5                  10                  15

Lys Ser Ile Cys Ser Leu Gly Cys Asp Leu Pro Gln Thr His Ser Leu
            20                  25                  30

Arg Asn Arg Arg Ala Leu Ile Leu Leu Ala Gln Met Gly Arg Ile Ser
        35                  40                  45

Pro Phe Ser Cys Leu Lys Asp Arg His Glu Phe Arg Phe Pro Glu Glu
    50                  55                  60

Glu Phe Asp Gly His Gln Phe Gln Lys Thr Gln Ala Ile Ser Val Leu
65                  70                  75                  80

His Glu Met Ile Gln Gln Thr Phe Asn Leu Phe Ser Thr Glu Asp Ser
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                85                  90                  95

Ser Ala Ala Trp Glu Gln Ser Leu Leu Glu Lys Phe Ser Thr Glu Leu
            100                 105                 110

Tyr Gln Gln Leu Asn Asp Leu Glu Ala Cys Val Ile Gln Glu Val Gly
        115                 120                 125

Val Glu Glu Thr Pro Leu Met Asn Glu Asp Phe Ile Leu Ala Val Arg
    130                 135                 140

Lys Tyr Phe Gln Arg Ile Thr Leu Tyr Leu Met Glu Lys Lys Tyr Ser
145                 150                 155                 160

Pro Cys Ala Trp Glu Val Val Arg Ala Glu Ile Met Arg Ser Phe Ser
                165                 170                 175

Phe Ser Thr Asn Leu Lys Lys Gly Leu Arg Arg Lys Asp
            180                 185


<210> SEQ ID NO 33
<211> LENGTH: 1109
<212> TYPE: DNA
<213> ORGANISM: Homo sapiens
<220> FEATURE:
<221> NAME/KEY: CDS
<222> LOCATION: (110)...(676)

<400> SEQUENCE: 33

gtatgttcac tatttaagac ctatgcacag agcaaagtct tcagaaaacc tagaggccac     60

ggttcaagtt acccacctca ggtagcctag tgatatttgc aaaatccca atg gcc ctg    118
                                                    Met Ala Leu
                                                     1

tcc ttt tct tta ctt atg gcc gtg ctg gtg ctc agc tac aaa tcc atc      166
Ser Phe Ser Leu Leu Met Ala Val Leu Val Leu Ser Tyr Lys Ser Ile
 5                  10                  15

tga tct ctg ggc tgt gat ctg cct cag acc cac acc ctg cgt aat agg      214
 *  Ser Leu Gly Cys Asp Leu Pro Gln Thr His Thr Leu Arg Asn Arg
     20                  25                  30

agg gcc ttg ata ctc ctg gga caa atg gga aga atc tct cct ttc tcc      262
Arg Ala Leu Ile Leu Leu Gly Gln Met Gly Arg Ile Ser Pro Phe Ser
 35                  40                  45                  50

tgc ctg aag gac aga cat gat ttc cga atc ccc cag gag gag ttt gat      310
Cys Leu Lys Asp Arg His Asp Phe Arg Ile Pro Gln Glu Glu Phe Asp
                55                  60                  65

ggc aac cag ttc cag aag gct caa gcc atc tct gtc ctc cat gag atg      358
Gly Asn Gln Phe Gln Lys Ala Gln Ala Ile Ser Val Leu His Glu Met
            70                  75                  80

atc cag cag acc ttc aat ctc ttc agc aca gag gac tca tct gct gct      406
Ile Gln Gln Thr Phe Asn Leu Phe Ser Thr Glu Asp Ser Ser Ala Ala
        85                  90                  95

tgg gaa cag agc ctc cta gaa aaa ttt tcc act gaa att tac cag caa      454
Trp Glu Gln Ser Leu Leu Glu Lys Phe Ser Thr Glu Ile Tyr Gln Gln
    100                 105                 110

ctg aat gac ctg gaa gca tgt gtg ata cag gag gtt ggg gtg gaa gag      502
Leu Asn Asp Leu Glu Ala Cys Val Ile Gln Glu Val Gly Val Glu Glu
115                 120                 125                 130

act ccc ctg atg aat gag gac tcc atc ctg gct gtg agg aaa tac ttc      550
Thr Pro Leu Met Asn Glu Asp Ser Ile Leu Ala Val Arg Lys Tyr Phe
                135                 140                 145

caa aga atc act ctt tat cta ata gag agg aaa tac agc cct tgt gcc      598
Gln Arg Ile Thr Leu Tyr Leu Ile Glu Arg Lys Tyr Ser Pro Cys Ala
            150                 155                 160

tgg gag gtt gtc aga gca gaa atc atg aga tcc ctc tcg ttt tca aca      646
Trp Glu Val Val Arg Ala Glu Ile Met Arg Ser Leu Ser Phe Ser Thr
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        165                 170                 175

aac ttg caa aaa aga tta agg agg aag gat tgaaaactgg ttcaacatgg       696
Asn Leu Gln Lys Arg Leu Arg Arg Lys Asp
    180                 185

caatgatcct gattgactaa tacattatct cacactttca tgagttcttc catttcaaag   756

actcacttct ataaccacga cgtgttgaat caaaattttc aaatgttttc agcagtgtaa   816

agaagtgtcg tgtatacctg tgcaggcact agtcctttac agatgaccat tctgatgtct   876

ctgttcatct tttgtttaaa tatttattta attatttta aaatttatgt aatatcatga    936

gtcgctttac attgtggtta atgtaacaat atatgttctt catatttagc caatatatta    996

atttcctttt tcattaaatt tttactatac aaaatttctt gtgtttgttt attctttaag   1056

ataaaatgcc aaggctgact ttacaacctg acttaaaaat agatgattta att          1109


<210> SEQ ID NO 34
<211> LENGTH: 169
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 34

Ser Leu Gly Cys Asp Leu Pro Gln Thr His Thr Leu Arg Asn Arg Arg
 1               5                  10                  15

Ala Leu Ile Leu Leu Gly Gln Met Gly Arg Ile Ser Pro Phe Ser Cys
            20                  25                  30

Leu Lys Asp Arg His Asp Phe Arg Ile Pro Gln Glu Glu Phe Asp Gly
        35                  40                  45

Asn Gln Phe Gln Lys Ala Gln Ala Ile Ser Val Leu His Glu Met Ile
    50                  55                  60

Gln Gln Thr Phe Asn Leu Phe Ser Thr Glu Asp Ser Ser Ala Ala Trp
65                  70                  75                  80

Glu Gln Ser Leu Leu Glu Lys Phe Ser Thr Glu Ile Tyr Gln Gln Leu
                85                  90                  95

Asn Asp Leu Glu Ala Cys Val Ile Gln Glu Val Gly Val Glu Glu Thr
            100                 105                 110

Pro Leu Met Asn Glu Asp Ser Ile Leu Ala Val Arg Lys Tyr Phe Gln
        115                 120                 125

Arg Ile Thr Leu Tyr Leu Ile Glu Arg Lys Tyr Ser Pro Cys Ala Trp
    130                 135                 140

Glu Val Val Arg Ala Glu Ile Met Arg Ser Leu Ser Phe Ser Thr Asn
145                 150                 155                 160

Leu Gln Lys Arg Leu Arg Arg Lys Asp
                165


<210> SEQ ID NO 35
<211> LENGTH: 188
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 35

Met Ala Leu Thr Phe Ala Leu Leu Val Ala Leu Leu Val Leu Ser Cys
 1               5                  10                  15

Lys Ser Ser Cys Ser Val Gly Cys Asp Leu Pro Gln Thr His Ser Leu
            20                  25                  30

Gly Ser Arg Arg Thr Leu Met Leu Leu Ala Gln Met Arg Arg Ile Ser
        35                  40                  45

Leu Phe Ser Cys Leu Lys Asp Arg His Asp Phe Gly Phe Pro Gln Glu
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    50                  55                  60

Glu Phe Gly Asn Gln Phe Gln Lys Ala Glu Thr Ile Pro Val Leu His
65                  70                  75                  80

Glu Met Ile Gln Gln Ile Phe Asn Leu Phe Ser Thr Lys Asp Ser Ser
                85                  90                  95

Ala Ala Trp Asp Glu Thr Leu Leu Asp Lys Phe Tyr Thr Glu Leu Tyr
            100                 105                 110

Gln Gln Leu Asn Asp Leu Glu Ala Cys Val Ile Gln Gly Val Gly Val
        115                 120                 125

Thr Glu Thr Pro Leu Met Lys Glu Asp Ser Ile Leu Ala Val Arg Lys
    130                 135                 140

Tyr Phe Gln Arg Ile Thr Leu Tyr Leu Lys Glu Lys Lys Tyr Ser Pro
145                 150                 155                 160

Cys Ala Trp Glu Val Val Arg Ala Glu Ile Met Arg Ser Phe Ser Leu
                165                 170                 175

Ser Thr Asn Leu Gln Glu Ser Leu Arg Ser Lys Glu
            180                 185


<210> SEQ ID NO 36
<211> LENGTH: 189
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 36

Met Ala Leu Pro Phe Ser Leu Met Met Ala Leu Val Val Leu Ser Cys
 1               5                  10                  15

Lys Ser Ser Cys Ser Leu Gly Cys Asn Leu Ser Gln Thr His Ser Leu
            20                  25                  30

Asn Asn Arg Arg Thr Leu Met Leu Met Ala Gln Met Arg Arg Ile Ser
        35                  40                  45

Pro Phe Ser Cys Leu Lys Asp Arg His Asp Phe Glu Phe Pro Gln Glu
    50                  55                  60

Glu Phe Asp Gly Asn Gln Phe Gln Lys Ala Gln Ala Ile Ser Val Leu
65                  70                  75                  80

His Glu Met Met Gln Gln Thr Phe Asn Leu Phe Ser Thr Lys Asn Ser
                85                  90                  95

Ser Ala Ala Trp Asp Glu Thr Leu Leu Glu Lys Phe Tyr Ile Glu Leu
            100                 105                 110

Phe Gln Gln Met Asn Asp Leu Glu Ala Cys Val Ile Gln Glu Val Gly
        115                 120                 125

Val Glu Glu Thr Pro Leu Met Asn Glu Asp Ser Ile Leu Ala Val Arg
    130                 135                 140

Lys Tyr Phe Gln Arg Ile Thr Leu Tyr Leu Met Glu Lys Lys Tyr Ser
145                 150                 155                 160

Pro Cys Ala Trp Glu Val Val Arg Ala Glu Ile Met Arg Ser Leu Ser
                165                 170                 175

Phe Ser Thr Asn Leu Gln Lys Arg Leu Arg Arg Lys Asp
            180                 185


<210> SEQ ID NO 37
<211> LENGTH: 189
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 37

Met Ala Leu Ser Phe Ser Leu Leu Met Ala Val Leu Val Leu Ser Tyr
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 1               5                  10                  15

Lys Ser Ile Cys Ser Leu Gly Cys Asp Leu Pro Gln Thr His Ser Leu
            20                  25                  30

Gly Asn Arg Arg Ala Leu Ile Leu Leu Ala Gln Met Gly Arg Ile Ser
        35                  40                  45

Pro Phe Ser Cys Leu Lys Asp Arg Pro Asp Phe Gly Leu Pro Gln Glu
    50                  55                  60

Glu Phe Asp Gly Asn Gln Phe Gln Lys Thr Gln Ala Ile Ser Val Leu
65                  70                  75                  80

His Glu Met Ile Gln Gln Thr Phe Asn Leu Phe Ser Thr Glu Asp Ser
                85                  90                  95

Ser Ala Ala Trp Glu Gln Ser Leu Leu Glu Lys Phe Ser Thr Glu Leu
            100                 105                 110

Tyr Gln Gln Leu Asn Asn Leu Glu Ala Cys Val Ile Gln Glu Val Gly
        115                 120                 125

Met Glu Glu Thr Pro Leu Met Asn Glu Asp Ser Ile Leu Ala Val Arg
    130                 135                 140

Lys Tyr Phe Gln Arg Ile Thr Leu Tyr Leu Thr Glu Lys Lys Tyr Ser
145                 150                 155                 160

Pro Cys Ala Trp Glu Val Val Arg Ala Glu Ile Met Arg Ser Leu Ser
                165                 170                 175

Phe Ser Thr Asn Leu Gln Lys Ile Leu Arg Arg Lys Asp
            180                 185


<210> SEQ ID NO 38
<211> LENGTH: 169
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 38

Ser Leu Gly Cys Asp Leu Pro Gln Thr His Ser Leu Gly Asn Arg Arg
 1               5                  10                  15

Ala Leu Ile Leu Leu Gly Gln Met Gly Arg Ile Ser Pro Phe Ser Cys
            20                  25                  30

Leu Lys Asp Arg His Asp Phe Arg Ile Pro Gln Glu Glu Phe Asp Gly
        35                  40                  45

Asn Gln Phe Gln Lys Ala Gln Ala Ile Ser Val Leu His Glu Met Ile
    50                  55                  60

Gln Gln Thr Phe Asn Leu Phe Ser Thr Glu Asp Ser Ser Ala Ala Trp
65                  70                  75                  80

Glu Gln Ser Leu Leu Glu Lys Phe Ser Thr Glu Ile Tyr Gln Gln Leu
                85                  90                  95

Asn Asp Leu Glu Ala Cys Val Ile Gln Glu Val Gly Val Glu Glu Thr
            100                 105                 110

Pro Leu Met Asn Glu Asp Ser Ile Leu Ala Val Arg Lys Tyr Phe Gln
        115                 120                 125

Arg Ile Thr Leu Tyr Leu Ile Glu Arg Lys Tyr Ser Pro Cys Ala Trp
    130                 135                 140

Glu Val Val Arg Ala Glu Ile Met Arg Ser Leu Ser Phe Ser Thr Asn
145                 150                 155                 160

Leu Gln Lys Arg Leu Arg Arg Lys Asp
                165


<210> SEQ ID NO 39
<211> LENGTH: 12
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<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Eco RI linker

<400> SEQUENCE: 39

catgaattca tg 12

<210> SEQ ID NO 40
<211> LENGTH: 14
<212> TYPE: DNA
<213> ORGANISM: Unknown
<220> FEATURE:
<223> OTHER INFORMATION: XbaI and EcoRI cleavage sites

<400> SEQUENCE: 40

tctagaattc tatg 14

<210> SEQ ID NO 41
<211> LENGTH: 14
<212> TYPE: DNA
<213> ORGANISM: Unknown
<220> FEATURE:
<223> OTHER INFORMATION: XbaI and EcoRI cleavage sites

<400> SEQUENCE: 41

catagaattc taga 14

<210> SEQ ID NO 42
<211> LENGTH: 11
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic deoxyoligonucleotides

<400> SEQUENCE: 42

aattcatgtg t 11

<210> SEQ ID NO 43
<211> LENGTH: 11
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic deoxyoligonucleotides

<400> SEQUENCE: 43

gatcacacat g 11

<210> SEQ ID NO 44
<211> LENGTH: 10
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: EcoRI to PstI convertor

<400> SEQUENCE: 44

aattctgcag 10

<210> SEQ ID NO 45
<211> LENGTH: 166
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens
<220> FEATURE:
<221> NAME/KEY: SITE
<222> LOCATION: (1)...(1)
<223> OTHER INFORMATION: Xaa = Methionine or Hydrogen

-continued

<400> SEQUENCE: 45

Xaa Cys Asp Leu Pro Gln Thr His Ser Leu Gly Ser Arg Arg Thr Leu
 1   5   10   15

Met Leu Leu Ala Gln Met Arg Lys Ile Ser Leu Phe Ser Cys Leu Lys
            20   25   30

Asp Arg His Asp Phe Gly Phe Pro Gln Glu Glu Phe Gly Asn Gln Phe
        35   40   45

Gln Lys Ala Glu Thr Ile Pro Val Leu His Glu Met Ile Gln Gln Ile
    50   55   60

Phe Asn Leu Phe Ser Thr Lys Asp Ser Ser Ala Ala Trp Asp Glu Thr
65   70   75   80

Leu Leu Asp Lys Phe Tyr Thr Glu Leu Tyr Gln Gln Leu Asn Asp Leu
                85   90   95

Glu Ala Cys Val Ile Gln Gly Val Gly Val Thr Glu Thr Pro Leu Met
            100   105   110

Lys Glu Asp Ser Ile Leu Ala Val Arg Lys Tyr Phe Gln Arg Ile Thr
        115   120   125

Leu Tyr Leu Lys Glu Lys Lys Tyr Ser Pro Cys Ala Trp Glu Val Val
    130   135   140

Arg Ala Glu Ile Met Arg Ser Phe Ser Leu Ser Thr Asn Leu Gln Glu
145   150   155   160

Ser Leu Arg Ser Lys Glu
                165

<210> SEQ ID NO 46
<211> LENGTH: 167
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens
<220> FEATURE:
<221> NAME/KEY: SITE
<222> LOCATION: (1)...(1)
<223> OTHER INFORMATION: Xaa = Methionine or Hydrogen

<400> SEQUENCE: 46

Xaa Cys Asp Leu Pro Gln Thr His Ser Leu Gly Asn Arg Arg Ala Leu
 1   5   10   15

Ile Leu Leu Ala Gln Met Arg Arg Ile Ser Pro Phe Ser Cys Leu Lys
            20   25   30

Asp Arg His Asp Phe Glu Phe Pro Gln Glu Glu Phe Asp Asp Lys Gln
        35   40   45

Phe Gln Lys Ala Gln Ala Ile Ser Val Leu His Glu Met Ile Gln Gln
    50   55   60

Thr Phe Asn Leu Phe Ser Thr Lys Asp Ser Ser Ala Ala Leu Asp Glu
65   70   75   80

Thr Leu Leu Asp Glu Phe Tyr Ile Glu Leu Asp Gln Gln Leu Asn Asp
                85   90   95

Leu Glu Val Leu Cys Asp Gln Glu Val Gly Val Ile Glu Ser Pro Leu
            100   105   110

Met Tyr Glu Asp Ser Ile Leu Ala Val Arg Lys Tyr Phe Gln Arg Ile
        115   120   125

Thr Leu Tyr Leu Thr Glu Lys Lys Tyr Ser Ser Cys Ala Trp Glu Val
    130   135   140

Val Arg Ala Glu Ile Met Arg Ser Phe Ser Leu Ser Ile Asn Leu Gln
145   150   155   160

Lys Arg Leu Lys Ser Lys Glu
                165

-continued

<210> SEQ ID NO 47
<211> LENGTH: 167
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens
<220> FEATURE:
<221> NAME/KEY: SITE
<222> LOCATION: (1)...(1)
<223> OTHER INFORMATION: Xaa = Methionine or Hydrogen

<400> SEQUENCE: 47

Xaa Cys Asp Leu Pro Gln Thr His Ser Leu Gly Asn Arg Arg Ala Leu
 1   5   10   15

Ile Leu Leu Gly Gln Met Gly Arg Ile Ser Pro Phe Ser Cys Leu Lys
            20   25   30

Asp Arg His Asp Phe Arg Ile Pro Gln Glu Glu Phe Asp Gly Asn Gln
        35   40   45

Phe Gln Lys Ala Gln Ala Ile Ser Val Leu His Glu Met Ile Gln Gln
    50   55   60

Thr Phe Asn Leu Phe Ser Thr Glu Asp Ser Ser Ala Ala Trp Glu Gln
65   70   75   80

Ser Leu Leu Glu Lys Phe Ser Thr Glu Leu Tyr Gln Gln Leu Asn Asp
                85   90   95

Leu Glu Ala Cys Val Ile Gln Glu Val Gly Val Glu Glu Thr Pro Leu
            100   105   110

Met Asn Glu Asp Ser Ile Leu Ala Val Arg Lys Tyr Phe Gln Arg Ile
        115   120   125

Thr Leu Tyr Leu Ile Glu Arg Lys Tyr Ser Pro Cys Ala Trp Glu Val
    130   135   140

Val Arg Ala Glu Ile Met Arg Ser Leu Ser Phe Ser Thr Asn Leu Gln
145   150   155   160

Lys Arg Leu Arg Arg Lys Asp
                165

<210> SEQ ID NO 48
<211> LENGTH: 167
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens
<220> FEATURE:
<221> NAME/KEY: SITE
<222> LOCATION: (1)...(1)
<223> OTHER INFORMATION: Xaa = Methionine or Hydrogen

<400> SEQUENCE: 48

Xaa Cys Asp Leu Pro Glu Thr His Ser Leu Asp Asn Arg Arg Thr Leu
 1   5   10   15

Met Leu Leu Ala Gln Met Ser Arg Ile Ser Pro Ser Ser Cys Leu Met
            20   25   30

Asp Arg His Asp Phe Gly Phe Pro Gln Glu Glu Phe Asp Gly Asn Gln
        35   40   45

Phe Gln Lys Ala Pro Ala Ile Ser Val Leu His Glu Leu Ile Gln Gln
    50   55   60

Ile Phe Asn Leu Phe Thr Thr Lys Asp Ser Ser Ala Ala Trp Asp Glu
65   70   75   80

Asp Leu Leu Asp Lys Phe Cys Thr Glu Leu Tyr Gln Gln Leu Asn Asp
                85   90   95

Leu Glu Ala Cys Val Met Gln Glu Glu Arg Val Gly Glu Thr Pro Leu
            100   105   110

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```
Met Asn Val Asp Ser Ile Leu Ala Val Lys Lys Tyr Phe Arg Arg Ile
        115                 120                 125

Thr Leu Tyr Leu Thr Glu Lys Lys Tyr Ser Pro Cys Ala Trp Glu Val
    130                 135                 140

Val Arg Ala Glu Ile Met Arg Ser Leu Ser Leu Ser Thr Asn Leu Gln
145                 150                 155                 160

Glu Arg Leu Arg Arg Lys Glu
                165
```

<210> SEQ ID NO 49
<211> LENGTH: 167
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens
<220> FEATURE:
<221> NAME/KEY: SITE
<222> LOCATION: (1)...(1)
<223> OTHER INFORMATION: Xaa = Methionine or Hydrogen

<400> SEQUENCE: 49

```
Xaa Cys Asp Leu Pro Gln Thr His Ser Leu Gly Asn Arg Arg Ala Leu
 1               5                  10                  15

Ile Leu Leu Ala Gln Met Gly Arg Ile Ser Pro Phe Ser Cys Leu Lys
            20                  25                  30

Asp Arg His Asp Phe Gly Phe Pro Gln Glu Glu Phe Asp Gly Asn Gln
        35                  40                  45

Phe Gln Lys Ala Gln Ala Ile Ser Val Leu His Glu Met Ile Gln Gln
    50                  55                  60

Thr Phe Asn Leu Phe Ser Thr Lys Asp Ser Ser Ala Thr Trp Glu Gln
65                  70                  75                  80

Ser Leu Leu Glu Lys Phe Ser Thr Glu Leu Asn Gln Gln Leu Asn Asp
                85                  90                  95

Met Glu Ala Cys Val Ile Gln Glu Val Gly Val Glu Glu Thr Pro Leu
            100                 105                 110

Met Asn Val Asp Ser Ile Leu Ala Val Lys Lys Tyr Phe Gln Arg Ile
        115                 120                 125

Thr Leu Tyr Leu Thr Glu Lys Lys Tyr Ser Pro Cys Ala Trp Glu Val
    130                 135                 140

Val Arg Ala Glu Ile Met Arg Ser Phe Ser Leu Ser Lys Ile Phe Gln
145                 150                 155                 160

Glu Arg Leu Arg Arg Lys Glu
                165
```

<210> SEQ ID NO 50
<211> LENGTH: 167
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens
<220> FEATURE:
<221> NAME/KEY: SITE
<222> LOCATION: (1)...(1)
<223> OTHER INFORMATION: Xaa = Methionine or Hydrogen

<400> SEQUENCE: 50

```
Xaa Cys Asn Leu Ser Gln Thr His Ser Leu Asn Asn Arg Arg Thr Leu
 1               5                  10                  15

Met Leu Met Ala Gln Met Arg Arg Ile Ser Pro Phe Ser Cys Leu Lys
            20                  25                  30

Asp Arg His Asp Phe Glu Phe Pro Gln Glu Glu Phe Asp Gly Asn Gln
        35                  40                  45

Phe Gln Lys Ala Gln Ala Ile Ser Val Leu His Glu Met Met Gln Gln
```

-continued

50 55 60

Thr Phe Asn Leu Phe Ser Thr Lys Asn Ser Ser Ala Ala Trp Asp Glu
65 70 75 80

Thr Leu Leu Glu Lys Phe Tyr Ile Glu Leu Phe Gln Gln Met Asn Asp
85 90 95

Leu Glu Ala Cys Val Ile Gln Glu Val Gly Val Glu Glu Thr Pro Leu
100 105 110

Met Asn Glu Asp Ser Ile Leu Ala Val Arg Lys Tyr Phe Gln Arg Ile
115 120 125

Thr Leu Tyr Leu Met Glu Lys Lys Tyr Ser Pro Cys Ala Trp Glu Val
130 135 140

Val Arg Ala Glu Ile Met Arg Ser Phe Ser Phe Ser Thr Asn Leu Gln
145 150 155 160

Lys Arg Leu Arg Arg Lys Asp
165

<210> SEQ ID NO 51
<211> LENGTH: 167
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens
<220> FEATURE:
<221> NAME/KEY: SITE
<222> LOCATION: (1)...(1)
<223> OTHER INFORMATION: Xaa = Methionine or Hydrogen

<400> SEQUENCE: 51

Xaa Cys Asp Leu Pro Gln Thr His Ser Leu Gly Asn Arg Arg Ala Leu
1 5 10 15

Ile Leu Leu Ala Gln Met Gly Arg Ile Ser Pro Phe Ser Cys Leu Lys
20 25 30

Asp Arg Pro Asp Phe Gly Leu Pro Gln Glu Glu Phe Asp Gly Asn Gln
35 40 45

Phe Gln Lys Thr Gln Ala Ile Ser Val Leu His Glu Met Ile Gln Gln
50 55 60

Thr Phe Asn Leu Phe Ser Thr Glu Asp Ser Ser Ala Ala Trp Glu Gln
65 70 75 80

Ser Leu Leu Glu Lys Phe Ser Thr Glu Leu Tyr Gln Gln Leu Asn Asn
85 90 95

Leu Glu Ala Cys Val Ile Gln Glu Val Gly Met Glu Glu Thr Pro Leu
100 105 110

Met Asn Glu Asp Ser Ile Leu Ala Val Arg Lys Tyr Phe Gln Arg Ile
115 120 125

Thr Leu Tyr Leu Thr Glu Lys Lys Tyr Ser Pro Cys Ala Trp Glu Val
130 135 140

Val Arg Ala Glu Ile Met Arg Ser Leu Ser Phe Ser Thr Asn Leu Gln
145 150 155 160

Lys Ile Leu Arg Arg Lys Asp
165

<210> SEQ ID NO 52
<211> LENGTH: 167
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens
<220> FEATURE:
<221> NAME/KEY: SITE
<222> LOCATION: (1)...(1)
<223> OTHER INFORMATION: Xaa = Methionine or Hydrogen

<400> SEQUENCE: 52

-continued

Xaa Cys Asp Leu Pro Gln Thr His Ser Leu Arg Asn Arg Arg Ala Leu
1 5 10 15

Ile Leu Leu Ala Gln Met Gly Arg Ile Ser Pro Phe Ser Cys Leu Lys
20 25 30

Asp Arg His Glu Phe Arg Phe Pro Glu Glu Glu Phe Asp Gly His Gln
35 40 45

Phe Gln Lys Thr Gln Ala Ile Ser Val Leu His Glu Met Ile Gln Gln
50 55 60

Thr Phe Asn Leu Phe Ser Thr Glu Asp Ser Ser Ala Ala Trp Glu Gln
65 70 75 80

Ser Leu Leu Glu Lys Phe Ser Thr Glu Leu Tyr Gln Gln Leu Asn Asp
85 90 95

Leu Glu Ala Cys Val Ile Gln Glu Val Gly Val Glu Glu Thr Pro Leu
100 105 110

Met Asn Glu Asp Phe Ile Leu Ala Val Arg Lys Tyr Phe Gln Arg Ile
115 120 125

Thr Leu Tyr Leu Met Glu Lys Lys Tyr Ser Pro Cys Ala Trp Glu Val
130 135 140

Val Arg Ala Glu Ile Met Arg Ser Phe Ser Phe Ser Thr Asn Leu Lys
145 150 155 160

Lys Gly Leu Arg Arg Lys Asp
165

<210> SEQ ID NO 53
<211> LENGTH: 166
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens
<220> FEATURE:
<221> NAME/KEY: SITE
<222> LOCATION: (1)...(1)
<223> OTHER INFORMATION: Xaa = Methionine or Hydrogen

<400> SEQUENCE: 53

Xaa Cys Asp Leu Pro Gln Thr His Ser Leu Gly Ser Arg Arg Thr Leu
1 5 10 15

Met Leu Leu Ala Gln Met Arg Arg Ile Ser Leu Phe Ser Cys Leu Lys
20 25 30

Asp Arg His Asp Phe Gly Phe Pro Gln Glu Glu Phe Gly Asn Gln Phe
35 40 45

Gln Lys Ala Glu Thr Ile Pro Val Leu His Glu Met Ile Gln Gln Ile
50 55 60

Phe Asn Leu Phe Ser Thr Lys Asp Ser Ser Ala Ala Trp Asp Glu Thr
65 70 75 80

Leu Leu Asp Lys Phe Tyr Thr Glu Leu Tyr Gln Gln Leu Asn Asp Leu
85 90 95

Glu Ala Cys Val Ile Gln Gly Val Gly Val Thr Glu Thr Pro Leu Met
100 105 110

Lys Glu Asp Ser Ile Leu Ala Val Arg Lys Tyr Phe Gln Arg Ile Thr
115 120 125

Leu Tyr Leu Lys Glu Lys Lys Tyr Ser Pro Cys Ala Trp Glu Val Val
130 135 140

Arg Ala Glu Ile Met Arg Ser Phe Ser Leu Ser Thr Asn Leu Gln Glu
145 150 155 160

Ser Leu Arg Ser Lys Glu
165

-continued

<210> SEQ ID NO 54
<211> LENGTH: 167
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens
<220> FEATURE:
<221> NAME/KEY: SITE
<222> LOCATION: (1)...(1)
<223> OTHER INFORMATION: Xaa = Methionine or Hydrogen

<400> SEQUENCE: 54

Xaa Cys Asp Leu Pro Gln Thr His Ser Leu Gly Asn Arg Arg Ala Leu
 1   5   10   15

Ile Leu Leu Gly Gln Met Gly Arg Ile Ser Pro Phe Ser Cys Leu Lys
            20   25   30

Asp Arg His Asp Phe Arg Ile Pro Gln Glu Glu Phe Asp Gly Asn Gln
        35   40   45

Phe Gln Lys Ala Gln Ala Ile Ser Val Leu His Glu Met Ile Gln Gln
    50   55   60

Thr Phe Asn Leu Phe Ser Thr Glu Asp Ser Ser Ala Ala Trp Glu Gln
65   70   75   80

Ser Leu Leu Glu Lys Phe Ser Thr Glu Ile Tyr Gln Gln Leu Asn Asp
                85   90   95

Leu Glu Ala Cys Val Ile Gln Glu Val Gly Val Glu Glu Thr Pro Leu
            100   105   110

Met Asn Glu Asp Ser Ile Leu Ala Val Arg Lys Tyr Phe Gln Arg Ile
        115   120   125

Thr Leu Tyr Leu Ile Glu Arg Lys Tyr Ser Pro Cys Ala Trp Glu Val
    130   135   140

Val Arg Ala Glu Ile Met Arg Ser Leu Ser Phe Ser Thr Asn Leu Gln
145   150   155   160

Lys Arg Leu Arg Arg Lys Asp
                165

<210> SEQ ID NO 55
<211> LENGTH: 167
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens
<220> FEATURE:
<221> NAME/KEY: SITE
<222> LOCATION: (1)...(1)
<223> OTHER INFORMATION: Xaa = Methionine or Hydrogen

<400> SEQUENCE: 55

Xaa Cys Asn Leu Ser Gln Thr His Ser Leu Asn Asn Arg Arg Thr Leu
 1   5   10   15

Met Leu Met Ala Gln Met Arg Arg Ile Ser Pro Phe Ser Cys Leu Lys
            20   25   30

Asp Arg His Asp Phe Glu Phe Pro Gln Glu Glu Phe Asp Gly Asn Gln
        35   40   45

Phe Gln Lys Ala Gln Ala Ile Ser Val Leu His Glu Met Met Gln Gln
    50   55   60

Thr Phe Asn Leu Phe Ser Thr Lys Asn Ser Ser Ala Ala Trp Asp Glu
65   70   75   80

Thr Leu Leu Glu Lys Phe Tyr Ile Glu Leu Phe Gln Gln Met Asn Asp
                85   90   95

Leu Glu Ala Cys Val Ile Gln Glu Val Gly Val Glu Glu Thr Pro Leu
            100   105   110

Met Asn Glu Asp Ser Ile Leu Ala Val Arg Lys Tyr Phe Gln Arg Ile

-continued

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        115                 120                 125

Thr Leu Tyr Leu Met Glu Lys Lys Tyr Ser Pro Cys Ala Trp Glu Val
    130                 135                 140

Val Arg Ala Glu Ile Met Arg Ser Leu Ser Phe Ser Thr Asn Leu Gln
145                 150                 155                 160

Lys Arg Leu Arg Arg Lys Asp
                165
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<210> SEQ ID NO 56
<211> LENGTH: 167
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens
<220> FEATURE:
<221> NAME/KEY: SITE
<222> LOCATION: (1)...(1)
<223> OTHER INFORMATION: Xaa = Methionine or Hydrogen

<400> SEQUENCE: 56

Xaa Cys Asn Leu Ser Gln Thr His Ser Leu Asn Asn Arg Arg Thr Leu
 1               5                  10                  15

Met Leu Met Ala Gln Met Arg Arg Ile Ser Pro Phe Ser Cys Leu Lys
            20                  25                  30

Asp Arg His Asp Phe Glu Phe Pro Gln Glu Glu Phe Asp Gly Asn Gln
        35                  40                  45

Phe Gln Lys Ala Gln Ala Ile Ser Val Leu His Glu Met Met Gln Gln
    50                  55                  60

Thr Phe Asn Leu Phe Ser Thr Lys Asn Ser Ser Ala Ala Trp Asp Glu
65                  70                  75                  80

Thr Leu Leu Glu Lys Phe Tyr Ile Glu Leu Phe Gln Gln Met Asn Asp
                85                  90                  95

Leu Glu Ala Cys Val Ile Gln Glu Val Gly Val Glu Glu Thr Pro Leu
            100                 105                 110

Met Asn Glu Asp Ser Ile Leu Ala Val Lys Lys Tyr Phe Gln Arg Ile
        115                 120                 125

Thr Leu Tyr Leu Met Glu Lys Lys Tyr Ser Pro Cys Ala Trp Glu Val
    130                 135                 140

Val Arg Ala Glu Ile Met Arg Ser Phe Ser Phe Ser Thr Asn Leu Gln
145                 150                 155                 160

Lys Arg Leu Arg Arg Lys Asp
                165


<210> SEQ ID NO 57
<211> LENGTH: 167
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens
<220> FEATURE:
<221> NAME/KEY: SITE
<222> LOCATION: (1)...(1)
<223> OTHER INFORMATION: Xaa = Methionine or Hydrogen

<400> SEQUENCE: 57

Xaa Cys Asp Leu Pro Gln Thr His Thr Leu Arg Asn Arg Arg Ala Leu
 1               5                  10                  15

Ile Leu Leu Gly Gln Met Gly Arg Ile Ser Pro Phe Ser Cys Leu Lys
            20                  25                  30

Asp Arg His Asp Phe Arg Ile Pro Gln Glu Glu Phe Asp Gly Asn Gln
        35                  40                  45

Phe Gln Lys Ala Gln Ala Ile Ser Val Leu His Glu Met Ile Gln Gln
    50                  55                  60
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-continued

Thr Phe Asn Leu Phe Ser Thr Glu Asp Ser Ser Ala Ala Trp Glu Gln
65 70 75 80

Ser Leu Leu Glu Lys Phe Ser Thr Glu Ile Tyr Gln Gln Leu Asn Asp
85 90 95

Leu Glu Ala Cys Val Ile Gln Glu Val Gly Val Glu Glu Thr Pro Leu
100 105 110

Met Asn Glu Asp Ser Ile Leu Ala Val Arg Lys Tyr Phe Gln Arg Ile
115 120 125

Thr Leu Tyr Leu Ile Glu Arg Lys Tyr Ser Pro Cys Ala Trp Glu Val
130 135 140

Val Arg Ala Glu Ile Met Arg Ser Leu Ser Phe Ser Thr Asn Leu Gln
145 150 155 160

Lys Arg Leu Arg Arg Lys Asp
165

<210> SEQ ID NO 58
<211> LENGTH: 167
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens
<220> FEATURE:
<221> NAME/KEY: SITE
<222> LOCATION: (1)...(1)
<223> OTHER INFORMATION: Xaa = Methionine or Hydrogen

<400> SEQUENCE: 58

Xaa Cys Asn Leu Ser Gln Thr His Ser Leu Asn Asn Arg Arg Thr Leu
1 5 10 15

Met Leu Met Ala Gln Met Arg Arg Ile Ser Pro Phe Ser Cys Leu Lys
20 25 30

Asp Arg His Asp Phe Glu Phe Pro Gln Glu Glu Phe Asp Gly Asn Gln
35 40 45

Phe Gln Lys Ala Gln Ala Ile Ser Val Leu His Glu Met Met Gln Gln
50 55 60

Thr Phe Asn Leu Phe Ser Thr Lys Asn Ser Ser Ala Ala Trp Asp Glu
65 70 75 80

Thr Leu Leu Glu Lys Phe Tyr Ile Glu Leu Phe Gln Gln Met Asn Asp
85 90 95

Leu Glu Ala Cys Val Ile Gln Glu Val Gly Val Glu Glu Thr Pro Leu
100 105 110

Met Asn Glu Asp Ser Ile Leu Ala Val Lys Lys Tyr Phe Gln Arg Ile
115 120 125

Thr Leu Tyr Leu Met Glu Lys Lys Tyr Ser Pro Cys Ala Trp Glu Val
130 135 140

Val Arg Ala Glu Ile Met Arg Ser Leu Ser Phe Ser Thr Asn Leu Gln
145 150 155 160

Lys Arg Leu Arg Arg Lys Asp
165

<210> SEQ ID NO 59
<211> LENGTH: 167
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens
<220> FEATURE:
<221> NAME/KEY: SITE
<222> LOCATION: (1)...(1)
<223> OTHER INFORMATION: Xaa = Methionine or Hydrogen

<400> SEQUENCE: 59

-continued

Xaa Cys Asp Leu Pro Gln Thr His Ser Leu Gly Asn Arg Arg Ala Leu
1 5 10 15

Ile Leu Leu Ala Gln Met Gly Arg Ile Ser Pro Phe Ser Cys Leu Lys
20 25 30

Asp Arg Pro Asp Phe Gly Leu Pro Gln Glu Glu Phe Asp Gly Asn Gln
35 40 45

Phe Gln Lys Thr Gln Ala Ile Ser Val Leu His Glu Met Ile Gln Gln
50 55 60

Thr Phe Asn Leu Phe Ser Thr Glu Asp Ser Ser Ala Ala Trp Glu Gln
65 70 75 80

Ser Leu Leu Glu Lys Phe Ser Thr Glu Leu Tyr Gln Gln Leu Asn Asn
85 90 95

Leu Glu Ala Cys Val Ile Gln Glu Val Gly Met Glu Glu Thr Pro Leu
100 105 110

Met Asn Glu Asp Ser Ile Leu Ala Val Arg Lys Tyr Phe Gln Arg Ile
115 120 125

Thr Leu Tyr Leu Thr Glu Lys Lys Tyr Ser Pro Ser Ala Trp Glu Val
130 135 140

Val Arg Ala Glu Ile Met Arg Ser Leu Ser Phe Ser Thr Asn Leu Gln
145 150 155 160

Lys Ile Leu Arg Arg Lys Asp
165

<210> SEQ ID NO 60
<211> LENGTH:
<212> TYPE:
<213> ORGANISM:

<400> SEQUENCE: 60

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<210> SEQ ID NO 61
<211> LENGTH:
<212> TYPE:
<213> ORGANISM:

<400> SEQUENCE: 61

000

<210> SEQ ID NO 62
<211> LENGTH: 15
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 62

Asp Asp Ala Ala Asp Leu Gln Pro Leu Gln His Lys Gly Leu Ile
1 5 10 15

<210> SEQ ID NO 63
<211> LENGTH: 8
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 63

Asp Pro Phe Arg Gln Ile Leu His
1 5

<210> SEQ ID NO 64
<211> LENGTH: 6
<212> TYPE: PRT

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<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 64

Thr Leu Pro Ala Ala Glu
 1   5

<210> SEQ ID NO 65
<211> LENGTH: 41
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 65

Pro Gly Ser Leu Cys Asp Val Glu Gly Trp Ser Gly Arg Asp Ser Pro
 1   5   10   15

Glu Glu Cys Gly Leu His Pro Gly Cys Glu Lys Ile Leu Ser Lys Asn
            20   25   30

His Ser Leu Ser Asp Lys Glu Glu Val
        35   40

<210> SEQ ID NO 66
<211> LENGTH: 29
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 66

Pro Leu Phe Leu Gly Gly Cys Gln Ser Arg Asn His Glu Ile Leu Leu
 1   5   10   15

Phe Met Asn Glu Leu Ala Gly Lys Ile Lys Glu Glu Gly
            20   25

<210> SEQ ID NO 67
<211> LENGTH: 52
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 67

Lys Val Gly Val Glu Glu Thr Pro Leu Arg Asn Val Asp Ser Ile Leu
 1   5   10   15

Ala Val Arg Lys Tyr Phe Gln Arg Ile Thr Leu Tyr Leu Thr Lys Lys
            20   25   30

Lys Tyr Ser Pro Cys Ser Trp Glu Ala Val Arg Ala Glu Ile Met Arg
        35   40   45

Ser Phe Ser Leu
    50

<210> SEQ ID NO 68
<211> LENGTH: 11
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 68

Thr Asn Leu Gln Glu Arg Leu Arg Arg Lys Glu
 1   5   10

<210> SEQ ID NO 69
<211> LENGTH: 19
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 69

Met Ala Leu Ser Phe Ser Leu Leu Met Ala Val Leu Val Leu Ser Tyr
 1   5   10   15

-continued

Lys Ser Ile

<210> SEQ ID NO 70
<211> LENGTH: 19
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 70

Met Ala Leu Ser Phe Ser Leu Leu Met Ala Val Leu Val Leu Ser Tyr
1 5 10 15

Lys Ser Ile

What is claimed is:

1. A DNA comprising a nucleotide sequence encoding a polypeptide of 165–166 amino acids having the amino acid sequence of a mature human leukocyte interferon unaccompanied by any corresponding presequence or portion thereof, wherein said nucleotide sequence further comprises an ATG immediately preceding the codon corresponding to the amino-terminal amino acid of said polypeptide.

2. The DNA according to claim **1**, in which the mature human leukocyte interferon has the amino acid sequence of SEQ ID NO:45, wherein the amino-terminal amino acid is cysteine.

3. The DNA according to claim **1**, in which the mature human leukocyte interferon has the amino acid sequence of SEQ ID NO:46, wherein the amino-terminal amino acid is cysteine.

4. The DNA according to claim **1**, in which the mature human leukocyte interferon has the amino acid sequence of SEQ ID NO:47, wherein the amino-terminal amino acid is cysteine.

5. The DNA according to claim **1**, in which the mature human leukocyte interferon has the amino acid sequence of SEQ ID NO:49, wherein the amino-terminal amino acid is cysteine.

6. The DNA according to claim **1**, in which the mature human leukocyte interferon has the amino acid sequence of SEQ ID NO:50, wherein the amino-terminal amino acid is cysteine.

7. The DNA according to claim **1**, in which the mature human leukocyte interferon has the amino acid sequence of SEQ ID NO:51, wherein the amino-terminal amino acid is cysteine.

8. The DNA according to claim **1**, in which the mature human leukocyte interferon has the amino acid sequence of SEQ ID NO:52, wherein the amino-terminal amino acid is cysteine.

9. The DNA according to claim **1**, in which the mature human leukocyte interferon has the amino acid sequence of SEQ ID NO:48, wherein the amino-terminal amino acid is cysteine.

10. The DNA according to claim **1**, in which the mature human leukocyte interferon has the amino acid sequence of SEQ ID NO:53, wherein the amino-terminal amino acid is cysteine.

11. The DNA according to claim **1**, in which the mature human leukocyte interferon has the amino acid sequence of SEQ ID NO:54, wherein the amino-terminal amino acid is cysteine.

12. The DNA according to claim **1**, in which the mature human leukocyte interferon has the amino acid sequence of SEQ ID NO:55, wherein the amino-terminal amino acid is cysteine.

13. The DNA according to claim **1**, in which the mature human leukocyte interferon has the amino acid sequence of SEQ ID NO:56, wherein the amino-terminal amino acid is cysteine.

14. The DNA according to claim **1**, in which the mature human leukocyte interferon has the amino acid sequence of SEQ ID NO:57, wherein the amino-terminal amino acid is cysteine.

15. The DNA according to claim **1**, in which the mature human leukocyte interferon has the amino acid sequence of SEQ ID NO:58, wherein the amino-terminal amino acid is cysteine.

16. The DNA according to claim **1**, in which the mature human leukocyte interferon has the amino acid sequence of SEQ ID NO:59, wherein the amino-terminal amino acid is cysteine.

17. A process for producing a polypeptide product, comprising culturing a bacterium transformed with a replicable bacterial expression vehicle comprising a DNA according to any of claims **1–16**, said vehicle capable of expressing in said bacterium a mature human leukocyte interferon having about 165–166 amino acids unaccompanied by any corresponding presequence or portion thereof.

18. A process for producing a polypeptide product, comprising culturing a bacterium transformed with a replicable bacterial expression vehicle comprising a DNA according to claim **2**, said vehicle capable of expressing in said bacterium a mature human leukocyte interferon having about 165–166 amino acids unaccompanied by any corresponding presequence or portion thereof.

19. A process for producing a polypeptide product, comprising culturing a bacterium transformed with a replicable bacterial expression vehicle comprising a DNA according to claim **10**, said vehicle capable of expressing in said bacterium a mature human leukocyte interferon having about 165–166 amino acids unaccompanied by any corresponding presequence or portion thereof.

20. A bacterium transformed with a DNA according to claims **1–16**.

21. A bacterium according to claim **20**, wherein the bacterium is *E. coli.*

22. A bacterium according to claim **20**, wherein the bacterium is *E. coli* K-12 strain 294.

* * * * *